PHOTOMEDICINE

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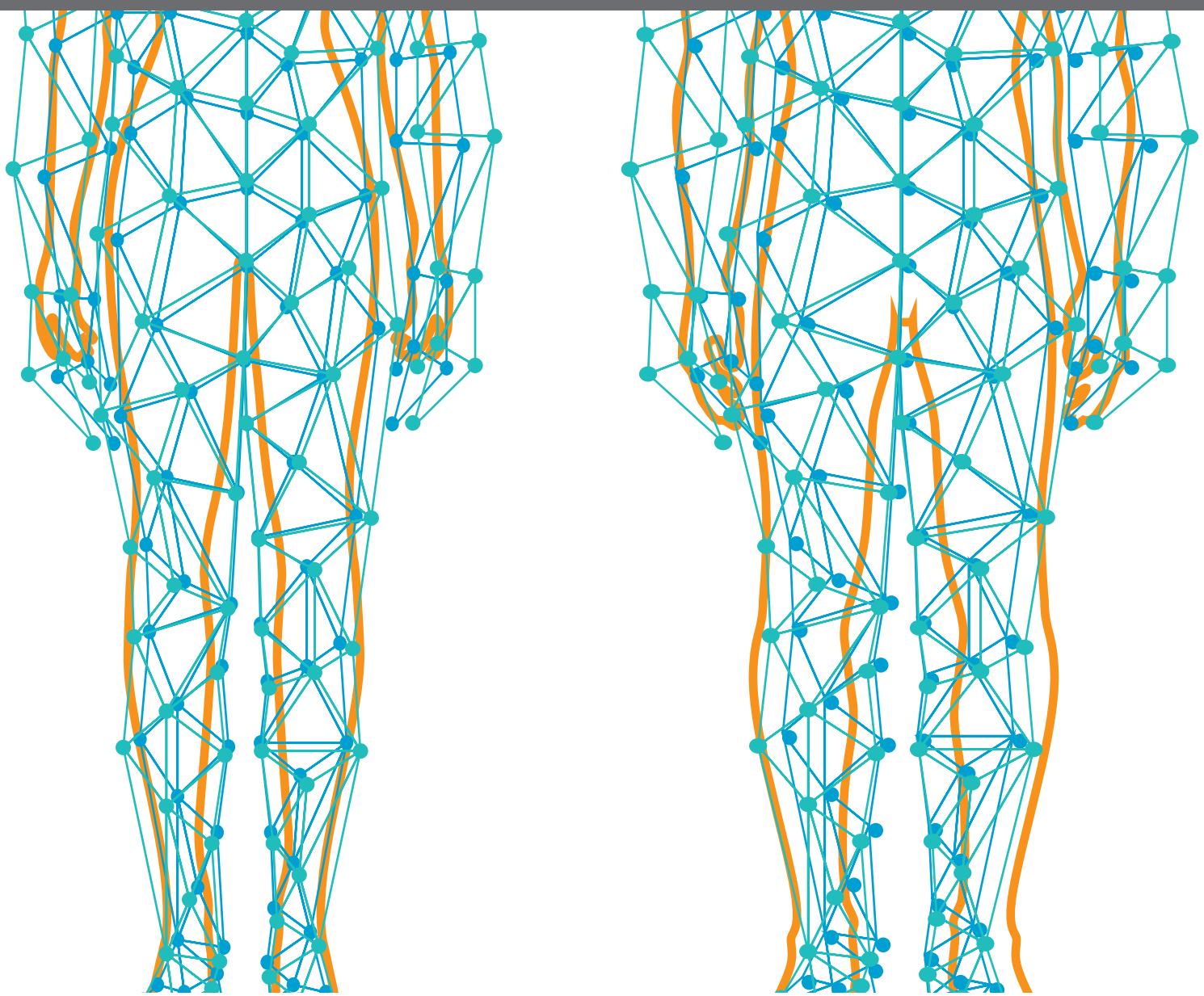

frontiers Research Topics

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PHOTOMEDICINE

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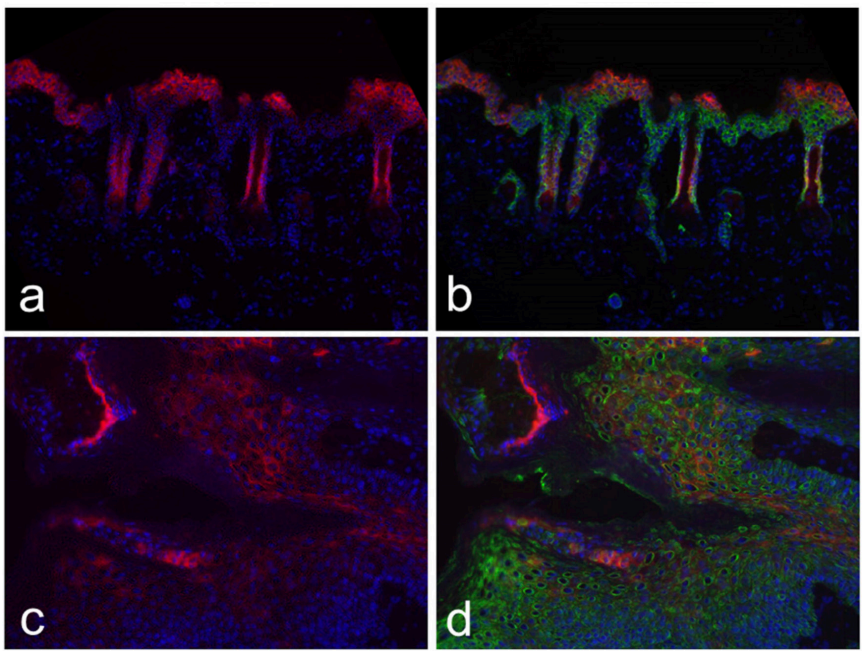

Image: Frank R. de Gruijl and Cornelis P. Tensen. Pathogenesis of Skin Carcinomas and a Stem Cell as Focal Origin. Front. Med. 5:165. doi: 10.3389/fmed.2018.00165

This Research Topic in Frontiers in Medicine offers a concise overview of the latest knowledge in the field of Photomedicine on therapeutic and preventive modalities, and UV pathogenesis of skin cancer. Photomedicine has revolutionized the treatment of inflammatory and neoplastic skin diseases over the last century since Niels Ryberg Finsen was awarded in 1903 the Nobel Prize "in recognition of his contribution to the treatment of diseases, especially lupus vulgaris, with concentrated light radiation, whereby he has opened a new avenue for medical science". From then on, the field has moved on in leaps and bounds to expand phototreatment to many inflammatory as well as neoplastic skin diseases.

With this Research Topic we aim to promote the field by giving the latest insights into Photomedicine's therapeutic modalities and molecular mechanisms in treatment of diseases of the skin and beyond, as well as prevention from photodermatoses and

carcinogenesis, as the dark sites of light. By engaging clinicians and scientists we intend to advance basic, clinical and translational research in the field of Photomedicine with its numerous facets.

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EDITORIAL
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Editorial: Photomedicine

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Keywords: ultraviolet radiation, phototherapy, immune suppression, microbiome, psoriasis, itch, carcinogenesis, photoprotection

Editorial on the Research Topic

Photomedicine

Photodermatology is the scientific discipline that deals with how sunlight or parts of it, in particular the ultraviolet (UV) band, affects the skin, our directly visible, frontier organ facing our environment. Although this discipline would appear well within the domain of our every-day experience, many of the basic processes involved are still not fully charted and understood. With regard to therapeutic approaches, the term of photomedicine has been coined, also because some of the effects of light go far beyond the skin and light administration is also used in medicine in general. This special issue aims to present a selection of topics to provide a bird's eye view of the field.

An area of broad public interest is UV protection. Sondenheimer and Krutmann discuss protection of the skin to wavelengths beyond UV by a novel generation of topical agents that boost protective mechanisms of the skin. Parrado et al. consider the possiblity of providing systemic protection by agents taken orally. The protection pertains to sunburn in the short term but to skin cancer in the long term. de Gruijl and Tensen present an overview of how our understanding of the UV pathogenesis of skin carcinoma has grown, in particular the plausible involvement of skin stem cells. And Arisi et al. delve into at times confusing body of data on how solar UV could contribute to raising melanomas, the most aggressive skin cancer.

The skin as a pivotal organ in immunity has become in many ways the essence of photodermatology. Photoimmunology is recognized as a distinguished field of research ever since the discovery of suppressive effects on cellular immunity from UV exposure. In this perspective, recent developments in phototherapy are discussed by Vieyra-Garcia and Wolf. Patra et al. present a novel view on the skin immune system in relation to the skin's microbiome and possible effect thereon from UV exposure; an evidently complicated but promising field of research. As a possibly related issue, Lembo and Raimondo present the advances that have been made in recent years in understanding the pathophysiology of polymorphic light eruption, the most common form of photodermatoses. In particular, a possible central role of an interplay between the immune system, its defense through antimicrobial peptides combined with an inadequate suppression of adaptive immunity against UV responses, on the one hand, and putative "photoantigen(s)" from UV-modified proteins released from (apoptotic) cells, on the other hand.

Besides, many forms and aspects of phototherapy are presented. Ibbotson provides an excellent perspective on the main indications for use of narrowband UVB (311–313 nm) and psoralen and UVA (PUVA) photochemotherapy and provides comparative information on these important dermatological treatments, which despite of the introduction of biologics continue to remain invaluable for many conditions such as psoriasis, atopic eczema, vitiligo, and cutaneous T cell lymphoma. Gambichler and Schmitz focus on the administration and therapeutic mechanisms of ultraviolet A1 (UVA1, 340–400 nm) for fibrosing conditions such as localized scleroderma,

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lichen sclerosus, systemic sclerosis, nephrogenic systemic fibrosis, and chronic graft-vs.-host-disease (GVHD) of the skin. In contrast, to the other phototherapeutic modalities UVA1 seems to induce changes in fibroblast cytokine production such as transforming growth factor- ß/Smad signaling and interleukin 6, leading to upregulation of collagenase activity, ultimately resulting in less tissue fibrosis. Legat thoroughly discusses the antipruritic effect of phototherapy. Pruritic skin diseases are another area in which phototherapy has remained a mainstay though new drugs, such as the anti-IL31RA antagonist nemolizumab among others, are emerging for itch treatment. It is fascinating to learn that UV may directly affect cutaneous sensory nerve fibers or, through blockage of mediator release (including IL-31) from skin-infiltrating cells, indirectly modulate nerve fiber function as well as the transmission of itch to the central nervous system, inducing the clinically evident antipruritic effect of phototherapy.

Last but not least, Cho et al. give a superb overview on the most complex form of phototherapy, extracorporeal photopheresis (ECP), from a technical point of view. The treatment is a therapeutic gold standard for patients with Sézary syndrome, a systemic form of T cell lymphoma that clinically presents with severe erythroderma. The disease is characterized by abnormal mononuclear cells, which appear in the skin, lymph nodes, and peripheral blood, where those cells and other cells are hit by ECP. Importantly, ECP is also a recommended second-line treatment in steroid-refractory GVHD. The induction of regulatory T cells seems to be the major driver of response in ECP-treated patients.

In sum, the collection of the papers of this special issue of photomedicine illustrates the beauty of the field and teaches how the different phototherapeutic modalities are useful and valuable for the patients, but also how their administration and mechanistic investigation leads to a better understanding of disease mechanisms, allowing ultimately the development of novel and advanced treatment strategies.

AUTHOR CONTRIBUTIONS

Both authors listed have made a substantial and direct contribution to the work, and approved it for publication.

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REVIEW
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From Early Immunomodulatory Triggers to Immunosuppressive Outcome: Therapeutic Implications of the Complex Interplay Between the Wavebands of Sunlight and the Skin

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Phototherapy is an efficient treatment for many cutaneous diseases that involve the activation of inflammatory pathways or the overgrowth of cells with aberrant phenotype. In this review, we discuss recent advances in photoimmunology, focusing on the effects of UV-based therapies currently used in dermatology. We describe the molecular responses to the main forms of photo(chemo)therapy such as UVB, UVA-1, and PUVA that include the triggering of apoptotic or immunosuppressive pathways and help to clear diseased skin. The early molecular response to UV involves DNA photoproducts, the isomerization of urocanic acid, the secretion of biophospholipids such as platelet activating factor (PAF), the activation of aryl hydrocarbon receptor and inflammasome, and vitamin D synthesis. The simultaneous and complex interaction of these events regulates the activity of the immune system both locally and systemically, resulting in apoptosis of neoplastic and/or benign cells, reduction of cellular infiltrate, and regulation of cytokines and chemokines. Regulatory T-cells and Langerhans cells, among other skin-resident cellular populations, are deeply affected by UV exposure and are therefore important players in the mechanisms of immunomodulation and the therapeutic value of UV in all its forms. We weigh the contribution of these cells to the therapeutic application of UV and how they may participate in transferring the direct impact of UV on the skin into local and systemic immunomodulation. Moreover, we review the therapeutic mechanisms revealed by clinical and laboratory animal investigations in the most common cutaneous diseases treated with phototherapy such as psoriasis, atopic dermatitis, vitiligo, and cutaneous T-cell lymphoma. Better understanding of phototherapeutic mechanisms in these diseases will help advance treatment in general and make future therapeutic strategies more precise, targeted, personalized, safe, and efficient.

Keywords: immunosupression, phototherapy, DNA damage, apoptosis, psoriasis, CTCL

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Sunlight and its wavebands profoundly affect the cellular physiology and dynamics of the skin. Exposure to ultraviolet radiation (UVR) leads in the short term to sunburn and tanning and in the long term to photoaging and carcinogenesis. However, it is also well known that UVR exposure can benefit patients with certain skin diseases including psoriasis, atopic dermatitis, and cutaneous T-cell lymphoma (CTCL). The initial triggers for these diverse effects of UVR include DNA damage (1); cis-to-trans urocanic acid (UCA) isomerization (2); formation of

active biophospholipids such as platelet activating factor (PAF) (3); and activation of aryl hydrocarbon receptor (AhR), inflammasome, and/or oxidative stress-related enzymes such as nitric oxygen synthase (NOS) (4, 5), Subsequent activation of apoptosis and mechanisms of local and systemic immunosuppression helps to counteract the effects of UV. After UV exposure, keratinocytes, melanocytes, and immune cells that reside in the skin, increase the release of cytokines such as TNF-α, IL-6, and IL-10 (6); chemokines such as CCL27 and IL-8 (7); and metabolic products such as vitamin D, that are involved in the onset of local and systemic effects of UV in complex regulatory loops. Langerhans cells (LC) and other dendritic cells as well as regulatory T-cells (Tregs) migrate in and out of the skin, thereby coordinating a series of crucial events for the establishment of an immunosuppressive microenvironment (8).

Last year, the many efforts to define the role of visible light in the complex interplay between UVR and living organisms received recognition when JC Hall, M Rosbash, and MW Young were awarded the Nobel Prize in medicine and physiology for their work on the genes that control circadian rhythm. Their work showed that proteins such as PER or TIM in fruit flies (9) and later CLOCK in mammals (10) accumulate during the night and degrade during the day in a self-regulatory feedback loop that establishes a neuronally regulated central clock-like system. In daytime, the skin is constantly exposed to UVR. The circadian rhythm pathways affect the skin's handling of UVR effects through cooperative or autonomous processes such as vitamin D synthesis, reactive oxygen species (ROS) production, DNA damage, cell senescence, and immunosuppression. One example of this influence on cutaneous dynamics is seen in mice whose food intake is restricted to certain times during the circadian cycle: alterations in biological clock genes like PER2 lead to a shift of up to 10% in the cutaneous transcriptome of animals under this food intake regime (11). Additionally, genes that mitigate photo-induced DNA damage like XPA are less active during the day in mice with high nocturnal food intake, resulting in prominent accumulation of cyclobutane pyrimidine dimers (CPD) induced by diurnal experimental UVR (11). Wound healing is also controlled by the circadian cycle; skin injuries suffered during the day heal faster than those suffered during the night due to a circadian control of actin polymerization regulated by CRY and PER2 proteins (12). Keratinocytes downregulate TIMP3, a metalloproteinase inhibitor linked to CLOCK upon UVR exposure, which in turn leads to an upregulation of MMP1, TNF-α, CXCL1, and IL-8 promoted by C/EBP (a CCAAT-enhancer binding protein) (13). This indicates that UVR affects tissue remodeling and inflammatory signaling pathways by modifying the transcriptional profile of keratinocytes. A recent study looking at the role of circadian proteins in psoriasis found that loss-of-function mutations in CLOCK lead to a less severe psoriatic phenotype in imiquimod-treated mice, whereas PER2 mutations lead to increased expression of IL-23R in γ/δ T-cells in the skin and a more severe psoriatic manifestation (14). If circadian proteins do indeed influence the severity of cutaneous diseases, then the effectiveness of phototherapy may also depend in part on circadian cycles. However, this has not yet been explored.

The physiologic reaction of the skin to UV exposure has been harnessed therapeutically. From the first attempts of Nobel laureate Niels Finsen to treat bacterial infections with UV (15) to the clinical approaches of today in which patients are exposed to UV radiation alone or in combination with photosensitizing agents (i.e., psoralens), (16) phototherapy has provided effective management of cutaneous diseases.

SENSING OF UV EXPOSURE AND TRIGGERING OF IMMUNOSUPPRESSION

DNA Damage

Insufficient DNA repair after UVR exposure leads to the accumulation of CPD, which in turn induces immunosuppression and can give rise to skin-tumorigenic gene mutations. The activation of DNA repair mechanisms is modulated in a TLR4/MyD88-dependent manner by the cleavage of the damage-recognition molecule PARP (17). The TLR4/MyD88 axis helps commit UV-exposed cells to apoptosis by activating caspase 3 (18). Experiments with $TLR4^{-/-}$ mice have shown that, after UV exposure, contact hypersensitivity (CHS) responses remain intact in these animals compared to wild type mice and the lymph nodes of $TLR4^{-/-}$ mice have fewer Tregs and lower production of IL-10 and TGF-β (19). This implicates TLR4 not only in the induction of apoptosis but also in the elicitation of immunosuppression after UV exposure. We have shown that the delivery of T4 endonuclease in liposomes to UV-irradiated skin leads to decreased secretion of IL-10 and TNF-α, suggesting that an increased DNA repair capacity can also increase resistance to UV-induced immunosuppression (**Figure 1A**) (6). Supplementation of IL-12 activates components of the nucleotide-excision repair complex that lower UV-induced DNA damage and prevent immunosuppression (20, 21). The reduced capacity for DNA repair after UV exposure in transplant patients or *in vitro* with immunosuppressive drugs indicate a two-way mechanism (22). Resident memory T-cells (T_{RM}) may be implicated in dealing with the effects of UVB. The main function of these cells is to provide surveillance and protection. They participate in wound healing by producing IGF-1 and immunity against pathogens like *Leishmania major* by producing IFN-γ (23, 24). After UV exposure, T_{RM} detect ATP release and increase the production of IL-17, leading to activation of TWEAK (an apoptosis inducer) and GADD45 (a damage-associated cell cycle arrest checkpoint protein), which in turn promote DNA repair (25, 26). Together, these findings highlight the tight interconnection between apoptosis and immunosuppression by means of innate and adaptive immunity and provide a rationale for UV-ameliorating therapies such as DNA repair enzyme supplementation.

Urocanic Acid Trans-isomerization

Urocanic acid is synthesized as trans-UCA from histidine in a reaction catalyzed by histidase. It accumulates in the skin at a high concentration (6 nmol/cm in humans) and after UV absorption is isomerized into cis-UCA and contributes to UV-induced immunosuppression (**Figure 1B**) (2). The treatment of keratinocytes with cis-UCA leads to upregulation of several genes

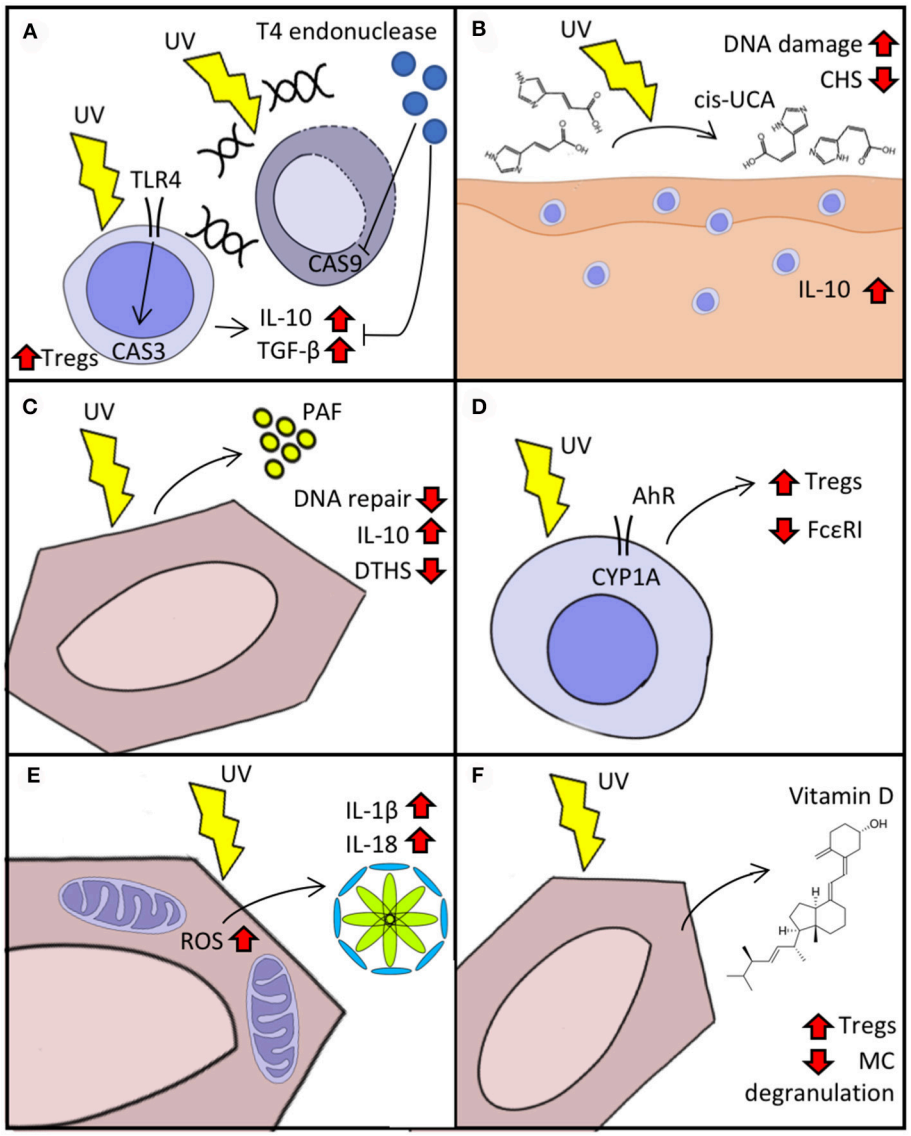

FIGURE 1 | Cellular response to UVR. **(A)** Immunosuppression in response to UV-induced DNA damage mediated by TLR4/MyD88. Delivery of T4 endonuclease decreases caspase (CAS) activation and the "production" of IL-10 and TGF-β. **(B)** Isomerization of urocanic acid (UCA) (trans to cis) after UV exposure increases IL-10 secretion and DNA damage and reduces contact hypersensitivity (CHS). **(C)** Keratinocyte secretion of platelet activating factor (PAF) augments immunosuppression and reduces DNA repair response to UV. **(D)** Activation of aryl hydrocarbon receptor (AhR) after UV reduces expression of FcεRI and boosts Treg activation. **(E)** Reactive oxygen species (ROS) production triggered by UV exposure activates inflammasome in keratinocytes. **(F)** Synthesis of vitamin D after UV exposure activates Tregs and decreases IgE-mediated "mast cell (MC)" degranulation.

that resemble the transcription profile induced by exposure to UVR, whereas treatment with trans-UCA does not lead to a shift of gene expression (2). Cytokines and proteins that participate in apoptosis, cell cycle arrest, and oxidative stress are upregulated after cis-UCA treatment. Notably, cis-UCA treatment in primary keratinocytes leads to activation of NF-κB and lipid peroxidation, suggesting a complex network of immunomodulatory-related gene transcription (27). The binding of cis-UCA and PAF to their respective receptors (5-HT2A and PAF receptor) contribute to sunburn cell formation, immune suppression, and skin cancer induction upon UVR exposure (28). Moreover, blockade of both cis-UCA and PAF but not vitamin D reduces UV-induced DNA damage in keratinocytes of mouse skin (29).

Platelet Activating Factor

Exposure to UVR elicits the secretion of PAF by keratinocytes, which in turn promotes the migration of mast cells into draining lymph nodes where they play an important role in immunosuppression (**Figure 1C**) (3). After PAF stimulation, mast cells undergo epigenetic modifications that increase their responsiveness to CXCR4 agonists. These modifications are mediated by increased expression of DNMT1/3b (members of

a DNA methyltransferase protein family) and p300 (a histone acetyltransferase) and decreased expression of HDAC2 (30). PAF also disrupts DNA-repair mechanisms upon UVR exposure by decreasing the expression of response elements such as MCPH1/BRIT-1 and ATR (31). We have reported that blockade of PAF receptor in mice treated with psoralen plus UVA (PUVA) leads to reduced IL-10 production, less delayed-type immune suppression in response to *Candida albicans*, and lower rates of keratinocyte apoptosis (32).

Aryl Hydrocarbon Receptor

CYP1A1 upregulation after UVR exposure implicates AhR in the skin's response to UV (4). AhR-knockout mice lack UVR-induced immunosuppression on CHS challenge (**Figure 1D**) (33). It has also been shown that AhR participates in the induction of Tregs during T-cell differentiation in the thymus and that certain AhR agonists such as TCDD activate Tregs in the skin and gut (34–36). Moreover, AhR not only participates in Treg-mediated UVR immunosuppression but also decreases the expression of the high affinity receptor for IgE (FcεRI) in LCs and upregulates immunosuppressive molecules such as IDO-1 (37). Atopic dermatitis in human patients is known to flare upon activation of FcεRI in LCs (38), suggesting that therapy with UVB may act by AhR-mediated downregulation of FcεRI.

Reactive Oxygen Species and Inflammasome

UV-irradiated skin shows immediate changes in a wide range of cellular processes. The biochemistry of keratinocytes and fibroblasts is rapidly redirected to produce ROS by increasing catalase activity and upregulating NOS (5). ROS activate various signaling pathways that involve stress-response factors, for example, the translocation of AP-1 and NF-κB, both of which are under the control of MAPKs that culminate in tissue remodeling and accelerated senescence (39).

ROS generation also activates inflammasome, a multiprotein intracellular oligomer responsible for initiating inflammatory responses by converting IL-1β and IL-18 into their active form and triggering inflammation-dependent cell death (pyroptosis) (**Figure 1E**) (40). UVB activates NLRP3 inflammasome in keratinocyte cells after sensing UVB-induced DNA damage (1). Yet, despite such inflammasome activation, the effects of UVR on the immune system are predominantly immunosuppressive. Upon UVB exposure, LCs emigrate from the epidermis in a process regulated by CXCR4 and α4-integrin (41, 42). After reaching draining lymph nodes, those LCs then become immunomodulatory intermediaries that promote Treg activation and produce IL-10 (43, 44).

Vitamin D

Vitamin D synthesis is initiated when UVB is absorbed by 7-dehydrocholesterol and converted to previtamin D3, which is then later converted to vitamin D3 (**Figure 1F**) (45). Most cells in the body express vitamin D receptor; hence, this molecule plays a role in numerous cellular processes including cell differentiation, cell growth inhibition, and immunomodulation (46). *In vitro* stimulation of mast cells with vitamin D suppresses IgE-mediated degranulation, while epicutaneous vitamin D administration reduces the magnitude of skin swelling in an IgE-mediated cutaneous anaphylaxis animal model (47). By promoting vitamin D3 synthesis and causing DNA damage such as CPD and 6-4PPs, UVB plays a dual role in carcinogenesis. For example, Ptch1-deficient mice are unable to produce vitamin D and demonstrate accelerated basal cell carcinoma-like tumor formation when exposed to UVR; this effect is reversed *in vivo* by exogenous supplementation of vitamin D (48). We have shown that polymorphic light eruption (PLE) patients have low levels of vitamin D in serum, however, prophylactic UVB treatment ameliorates PLE symptoms and increases vitamin D serum levels (49). A clinical trial evaluating the preventive properties of calcipotriol (a vitamin D analog) in 13 PLE patients showed that, 1 week of topical treatment with calcipotriol reduced the photoprovocative effect of simulated sun exposure and decreased severity disease score in PLE lesional skin (50).

LANGERHANS CELL AND REGULATORY T-CELLS ARE THE MAIN ORCHESTRATORS OF UV-INDUCED IMMUNOSUPPRESSION

LCs are a subset of dendritic cells that link the innate and adaptive immune systems by their role in priming T-cell responses upon antigen uptake. After UVR exposure, these cells migrate out of the skin and undergo changes that make them inducers of tolerance and immunosuppression (51). Compensatory mechanisms are activated after UVR exposure to repopulate the skin with LCs and rapidly recruit monocytes from blood (52). The transitory depletion of LCs is counteracted by the early recruitment of $CD14^+$ monocytes (after 24 h of UVB exposure) and subsequent mobilization of two inflammatory subsets of dendritic cells ($CD1a^{low}CD207^-$ and $CD1^{low}CD207^+$ at day 1 and 4 respectively) from blood circulating cells (53). Cells of the CD11b-type $Langerin^-$ phenotype are important players in the adaptive response to UVB. After irradiation, they upregulate the expression of CD86 that leads to antigen-free proliferation of Tregs and promotes the transcription of genes associated with immunotolerance (54). The skin is also populated by $CD103^-$ dendritic cells that upon UVR exposure migrate into lymph nodes and induce Treg activation by the production of retinoic acid (55). Mice depleted of LCs fail to suppress CHS reactions, indicating that these cells are major players in UV induction of Tregs. This suggests that the main function of LCs is not to promote immune responses but to desensitize the skin to UV exposure (56). UVR exposure not only drives the expansion of Tregs but also restores suppressive function by inducing demethylation of the Treg genome and thereby promoting gene transcription that counteracts inflammation in skin diseases such as psoriasis (57, 58). Moreover, Treg numbers in skin increase by up to 50–60% after UV irradiation and remain in high numbers for 2 weeks after irradiation. Indeed, the impact of UV is not restricted to the skin; mice exposed to UV have CPD-positive cells in their lymph nodes for at least 4 days after exposure (59) and Tregs isolated from the blood of UV-exposed animals have

TABLE 1 | Wavebands associated with key molecular events in UV-exposed tissue.

Molecular event	Causative wavebands (peak wavelength)
CPD	UVB (300) (63)
8-MOP photoadducts	UVA (329 nm), (64)
ROS production	UVA, UVA-1, PUVA (5)
Urocanic acid isomerization	UVB (280–310 nm) (65, 66)
Vitamin D synthesis	UVB (297 nm) (67)
PAF and PAF-like molecules	UVB, UVA, PUVA (32, 68)
Inflammasome activation	UVB (1)

a CpG hypomethylation fingerprint indicating that these Tregs were exposed to light (58). It seems that the immunosuppression triggered by UVR is in fact an adaptive response to mitigate the strong reaction of the immune system to the release of damage-associated molecular patterns (DAMPs) in favor of the repair and remodeling of damaged tissue as seen in animal models of brain injury and other trauma (60, 61). These observations suggest that the physiological immunosuppressive response to UVB that promotes the recovery of cells and damaged tissue can also be harnessed therapeutically to inflammatory diseases in body sites not exposed to UVR and therefore not only to cutaneous diseases.

HOW DOES PHOTOTHERAPY WORK?

Photo(chemo)therapy is a first-line treatment for skin diseases of diverse etiology, including benign conditions such as psoriasis, atopic dermatitis, vitiligo, and urticaria pigmentosa (a form of mastocytosis) as well as neoplastic disorders such as mycosis fungoides. It is also used prophylactically in certain photodermatoses like PLE. Though the high efficacy of phototherapy in these diseases has long been appreciated, the exact therapeutic mechanisms have not been fully understood until now and may depend upon the type of disease for which it is prescribed. The penetration depth of UV light increases with its wavelength. Whereas most of the photons of the UVB spectrum are absorbed in the epidermis, ∼30% of UVA photons do reach the upper layers of the dermis (62). The initial molecular events occurring after exposure to the different wavebands and treatments are depicted in **Table 1** and include CPD formation, ROS production, UCA isomerization, vitamin D synthesis, PAF secretion, and inflammasome activation (32, 63–65, 67, 68). The phototherapeutic modalities, including UVB, UVA, and PUVA, are known for their proapototic and immunomodulatory properties, which may account for their therapeutic efficacy either alone or in combination (8).

In particular, PUVA depletes activated $CD3^+$ cells from lesional psoriatic skin by the induction of apoptosis (69, 70). The majority of $CD3^+$ cells produce IL-17, a cytokine with a central role in psoriasis (71). Notably, PUVA and 311 nm UVB suppress the IL-17/IL-23 axis in both animal models and patients (72–76). Given the major role that these cells play in psoriasis pathophysiology, phototherapy's effect on them might explain (at least partially) its efficacy. But what is the fate of these activated T-cells? Are they directly eliminated by apoptosis or are they hampered by the complex immunomodulatory effects of phototherapy? Does the induction of Tregs (triggered by redundant upstream events including DNA and membrane damage as well cis-UCA formation and AhR activation) with immunosuppressive function diminish the number or the activity of those cells in skin? This is seen in psoriasis patients in whom bath PUVA therapy restores Treg functionality (77). Along this line, we have shown that CTLA-4 blockade abolishes the therapeutic effect of PUVA in a psoriasis mouse model (72). However, the systemic effect of phototherapy on the immune system and on Tregs seems therapeutically insufficient since psoriasis (78) and CTCL (79) lesions are cleared only on exposed body sites. This suggests that phototherapy must exert an additional direct local effect on keratinocytes, LCs, and/or lymphocytes among other players in the pathophysiology of those diseases, thereby allowing a local cell-to-cell interaction (between Tregs and pro-inflammatory effector T-cells) that leads to therapeutic response. For instance, on the local level, PUVA contributes to the normalization of the mTOR pathway upregulated in psoriasis (80). The systemic effect of PUVA or UVB in this disease may be completely independent of locally active mechanisms; for instance, serotonin signaling has been shown to play a crucial role in immune suppression but not inflammation or apoptosis in PUVA-exposed skin in a mouse model (81). A controversial computational model of psoriatic epidermis indicates that apoptosis of stem and transit amplifying cells after exposure to 311 nm UVB alone may be sufficient to clear lesional skin, suggesting that direct keratinocyte apoptosis is a key therapeutic mechanism (82). Moreover, psoriatic lesions clinically cleared after phototherapy contain residual oligoclonal T-cell populations that share features of T_{RM} and are capable of producing IL-17. These cells are likely responsible for the initiation of recurrent flares in the same body locations, implying that clinical resolution after phototherapy does not depend on depletion of cells with a dysregulated phenotype (83). In any case, the difficulty inherent in evaluating the roles of direct apoptosis and immunosuppression independently of each other highlights the need to investigate and compare phototherapy against other therapeutic approaches that induce one effect or the other.

In atopic dermatitis, phototherapy may work by strengthening the skin barrier function of lesional skin, shifting the expression of epidermal proteins like filaggrin, loricrin, and involucrin (84), augmenting levels of AMPs, (85) and shifting the microbiome diversity, among other effects (86, 87). In vitiligo, 311 UVB and PUVA directly stimulate the proliferation of melanocytes and by inducing Tregs help overcome the autoimmune pathophysiology of this disease by controlling cellular mediated cytotoxicity against pigment-producing cells (88). In mastocytosis, phototherapy might act by direct cytotoxicity against activated mast cells and by stabilizing mast cells, thus inhibiting them from releasing soluble proinflammatory mediators such as histamine (89). In graft vs. host disease (GVHD), the predominant mechanism of action may be immunomodulation by downregulating the activity of grafted cells against the host (90). The effect of OVB in pruritus remains entirely elusive at the moment; however, a halfside comparison

study implied a systemic effect, since treatment reduced pruritus not only on the irradiated body half but also to an equal degree on the unirradiated side (91). UVB-induced reduction of systemic levels of pro-pruritic IL-31 may be involved (92).

In the prophylaxis of photodermatoses such as PLE, phototherapy may act by inducing melanization in the skin, increasing vitamin D levels, restoring the susceptibility of the skin to respond to UV by depleting LCs and allowing infiltration of neutrophils, restoring the abnormal chemotactic potential of neutrophils, and increasing the number of peripheral Tregs to overcome the impaired immunosuppressive function of these cells (93, 94). Moreover, recent work has indicated that mast cells play a crucial role in countering itch by inducing phototolerance after photohardening treatment with increased numbers of Tregs in blood (95–97).

The efficacy of phototherapy in the most common form of CTCL, mycosis fungoides (MF), depends on the severity of the disease and on the type of presenting lesions. UVB has a high success rate in patients with patch-stage lesions, whereas PUVA is also effective in patients with plaque- and even early tumor-stage lesions. This differential response may be attributed to the lower penetration capacity of UVB compared to UVA as used in PUVA photochemotherapy. Alternatively, PUVA may induce longer lasting photoproducts than UVB does, resulting in a sustained downstream immunosuppressive cascade. Notably, phototherapy with both PUVA or UVB is effective not only in MF but also in lymphomatoid papulosis (LyP) (98), a disease that sometimes coexists with MF and is characterized by papules and nodules with deep skin infiltration up to 1 cm or more; however, these light treatments only directly reach the infiltrating cells in the most superficial layers but not those in the diseased deep tissue. The immunosuppressive microenvironment induced by phototherapy in the upper layers of the skin may be sufficient to deplete infiltrating cells in LyP and/or prevent the occurrence of new lesions in this intermittent disease.

Although broad band UVB, narrow band UVB, and oral or topical PUVA lead to different photoproducts at the DNA level (CPD vs. psoralen-DNA photoadducts) and produce overlapping molecular events (such as PAF and PAF like molecules) (**Table 1**), they have similar downstream effects including the induction of apoptosis and the downregulation of immune responses (including the induction of Tregs locally and systemically). In contrast, exposure to UVA and UVA1 (340–400 nm) mainly leads to oxidative alterations at the DNA and membrane level and elicits cellular responses such as the induction of MMPs and collagenase, mediators that are important particularly in UVA1's therapeutic action in fibrotic skin conditions including morphea and sclerodermic chronic GVHD (99). This may be due to the downregulation of TGFβ signaling transducers in the skin after UVA1 exposure (100).

CONCLUDING REMARKS

After more than 100 years of using simple artificial UV light therapeutically, beginning with the pioneering work of Nobel laureate Finsen in treating cutaneous tuberculosis, diverse photo(chemo)therapeutic modalities have evolved to treat a wide spectrum of skin diseases and to prevent photodermatoses. During this evolutionary process, photo(chemo)therapy has offered avenues to better understand disease and therapeutic mechanisms and provided a large body of evidence for refining therapeutic strategies in the future. In this context, our research with PUVA has led us to realize the potential role of IL-9 in psoriasis and CTCL. PUVA reduces levels of IL-9 and IL-17 in both the TGFβ transgenic and imiquimod psoriasis mouse model (72, 75). The blockade of IL-9 (101) or IL-17 (72) reduced the psoriatic phenotype of these mice. Meanwhile, IL-17 antibody blockers have reached the market and are currently considered the most powerful anti-psoriatic treatment. And now, in light of evidence that PUVA also downregulates IL-9 in CTCL patients and that anti-IL9 treatment reduces tumor growth in a CTCL mouse model (102), IL-9 targeting has become a promising therapeutic intervention in patients with CTCL. These and other advances in the understanding of phototherapeutic mechanisms in inflammatory and neoplastic diseases will help to make therapeutic strategies more precise, targeted, personalized, safe, and efficient.

AUTHOR CONTRIBUTIONS

PV-G and PW conceived the ideas and drafted the manuscript. PV-G drafted the figure. Both authors revised and approved the final version of the manuscript for publication.

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PERSPECTIVE
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A Perspective on the Use of NB-UVB Phototherapy vs. PUVA Photochemotherapy

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Narrowband UVB (NB-UVB) phototherapy and psoralen-UVA (PUVA) photochemotherapy are widely used phototherapeutic modalities for a range of skin diseases. The main indication for NB-UVB and PUVA therapies is psoriasis, and other key diagnoses include atopic eczema, vitiligo, cutaneous T-cell lymphoma (CTCL), and the photodermatoses. The decision on choice of phototherapy is important and NB-UVB is usually the primary choice. NB-UVB phototherapy is a safe and effective therapy which is usually considered when topical agents have failed. PUVA requires prior psoralen sensitization but remains a highly effective mainstay therapy, often used when NB-UVB fails, there is rapid relapse following NB-UVB or in specific indications, such as pustular or erythrodermic psoriasis. This review will provide a perspective on the main indications for use of NB-UVB and PUVA therapies and provide comparative information on these important dermatological treatments.

Keywords: UVB, PUVA therapy, phototherapy, skin diseases, psoriasis, eczema, vitiligo

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INTRODUCTION

Narrowband UVB (NB-UVB) phototherapy and psoralen-UVA (PUVA) photochemotherapy are widely used light-based treatments for a range of diverse skin diseases and can be highly effective, well-tolerated, safe, cost-saving, and reduce the need for topical therapies (1–6). The main indication for NB-UVB or PUVA is psoriasis (7) but other mainstay indications include atopic dermatitis or dermatitis of other cause, vitiligo, cutaneous T-cell lymphoma (CTCL), and a range of other conditions, including the photodermatoses, pityriasis rubra pilaris, urticaria, aquagenic pruritus, urticaria pigmentosum, pityriasis lichenoides, lichen planus, granuloma annulare, alopecia areata, and graft vs. host disease (2, 3, 5, 6) (**Table 1**).

If topical treatments fail to establish adequate control of disease then a light-based therapy would be a next appropriate treatment choice and in most instances NB-UVB would be selected as the primary phototherapeutic option. However, in certain diseases such as erythrodermic or pustular psoriasis, pityriasis rubra pilaris, or plaque stage CTCL, PUVA would be the desired option (5).

I am going to provide my opinion and perspective on the relative uses of NB-UVB and PUVA for a range of diseases, with particular emphasis on psoriasis as the predominant indication for a UV-light based therapy and with briefer mention on the salient points relative to the use of NB-UVB and PUVA in other conditions. I am restricting my review to NB-UVB and PUVA and am not including BB-UVB or UVA1 phototherapies.

TABLE 1 | Key indications for NB-UVB or PUVA.

Psoriasis
Pustular or erythrodermic*
Eczema – atopic or other type
Vitiligo
Cutaneous T-cell lymphoma
Patch
Plaque*
Photodermatoses
Polymorphic light eruption, actinic prurigo, solar urticaria, hydroa vacciniforme, erythropoietic protoporphyria
Chronic actinic dermatitis*
Urticaria
Urticaria pigmentosa
Aquagenic pruritus
Mastocytoses
Generalised pruritus
For example secondary to cholestasis or uraemia
Pityriasis lichenoides chronica
Lichen planus
Granuloma annulare
Graft vs. host disease
Alopecia areata*
Pityriasis rubra pilaris*
Hand & foot eczema*
Palmoplantar pustulosis*

**Consider PUVA in preference to UVB.*

BACKGROUND

UVB was introduced into increasingly widespread and routine use following developmental work in the 1980s (8–11). NB-UVB phototherapy reduces the need for topical therapies (1) and is a cost effective (12) and safe treatment, which involves repeated controlled delivery of the narrowband region of the UVB spectrum centered on 311 nm (4, 6). The main acute adverse effects of NB-UVB are erythema and induction of photosensitivity diseases, such as polymorphic light eruption (PLE). However, although the risk of erythemal episodes may be increased by concomitant phototoxic drugs (13, 14), this can be minimized by undertaking a baseline minimal erythema dose (MED) and establishing treatment protocols based on an individual's MED (15). This also allows any unsuspected abnormal photosensitivity diseases to be detected, in particular solar urticaria or chronic actinic dermatitis (CAD). Induction of PLE may occur during a treatment course but generally can be accommodated via dose adjustments and judicious use of topical corticosteroid, without the need to stop NB-UVB (16). Other uncommon side-effects, such as psoriatic lesional blistering, occasionally occur but generally treatment is very well-tolerated (17, 18). Importantly, NB-UVB can be safely used in children and in pregnancy and long-term studies to date do not indicate a significantly increased risk of skin cancer over an age- and sex-matched control population who have not received UVB phototherapy (19–21).

PUVA photochemotherapy is delivered using psoralen administration via either systemic (8-methoxypsoralen or 5-methoxypsoralen) or topical (usually now 8-methoxypsoralen as bath, soak, gel, cream, or lotion) routes (5). The mechanism of action of PUVA is quite distinct from that of UVB or of UVA alone, with PUVA inducing a delayed erythemal reaction peaking around 96 h after irradiation of psoralen-sensitized skin (22–27). This contrasts with the peak time for development of erythema after NB-UVB exposure of 12–24 h (28). Treatment is thus logistically slightly more of a challenge as psoralen sensitization is required. With systemic PUVA, appropriate skin and eye protection must be used for 24 h after psoralen ingestion. Oral 8-methoxypsoralen may cause some gastrointestinal upset, although switching to 5-methoxypsoralen minimizes this adverse effect and of course this is not an issue with topical PUVA. However, PUVA treatment can be highly effective and very safely administered in any Dermatology Department with a significantly sized Phototherapy Unit.

With the exception of less common adverse effects such as PUVA pain, treatment is otherwise usually well-tolerated (5). Undoubtedly, there is a longer term risk of skin carcinogenesis with high numbers of PUVA exposures (19, 29–37), but the risks can be minimized by vigilance, limitation of lifetime numbers of PUVA exposures, and avoidance of the use of maintenance PUVA where possible. As with all therapeutic approaches, benefit, and risk must be evaluated and it is important that PUVA is kept firmly in the range of treatment options as it can be highly effective, resulting in clearance, and marked improvement in quality of life for patients with psoriasis and a variety of other diseases.

It is essential that adequate governance is ensured for the safe delivery of both NB-UVB and PUVA therapies. In Scotland we have established the National Managed Clinical Network for phototherapy (Photonet; www.photonet.scot.nhs.uk), which employs a central database (Photosys), enabling standardization of treatment protocols, recording of treatment parameters, and outcomes and facilitating linkage studies to ascertain longer-term risks of treatment, notably skin cancer risk (20, 21). This has been an invaluable asset to allow standardization of phototherapy services in Scotland and delivery of effective and safe treatment for patients. This approach is now being adopted in England and has important roles in delivery of optimized safe care.

PSORIASIS

The main indication for any light-based therapy is psoriasis, and for the reasons highlighted in terms of practicalities and ease of treatment and its safety and potential for use in children and pregnancy, NB-UVB phototherapy would usually be the light-based therapy of choice, with high clearance rates achieved for chronic plaque psoriasis (6, 38–40).

In an initial controlled comparative half-body study in 10 patients with widespread psoriasis, no significant difference in efficacy was seen between twice weekly NB-UVB or systemic PUVA (41) and this observation was also reported

in a separate intra-individual open non-randomized controlled paired comparison study of three times weekly NB-UVB and PUVA, with no significant difference in efficacy seen between the treatment arms. However, there was a trend to superior efficacy with PUVA and this was particularly evident for patients with a higher baseline PASI score (42), possibly suggestive of a role for PUVA in more severe psoriasis or relapsing psoriasis, although given the convenience of NB-UVB this would generally be the preferred initial approach. In a separate inter-individual study of 100 patients with psoriasis, twice weekly PUVA was superior in efficacy to twice-weekly NB-UVB, with 35% of patients still being clear at 6 months after completion of PUVA, compared with only 12% after NB-UVB (43). These findings are supported by those of a separate study in which 93 patients with chronic plaque psoriasis were randomized to receive either twice-weekly oral PUVA or twice-weekly NB-UVB, resulting in 84% achieving clearance with PUVA compared with significantly lower clearance rates (65%) with NB-UVB and shorter remission, as 6 months after treatment 68% of those treated with PUVA were still in remission, compared with only 35% of patients treated with NB-UVB (44). Of note, lower clearance rates were achieved in patients of skin phototype V and VI, with only 24% achieving clearance, although baseline psoriasis severity was not a determinant of response in this study (44). However, high efficacy rates have been reported in patients of higher skin phototypes (IV and V), with 81–82% of patients showing marked improvement with three times weekly 8-MOP PUVA or NB-UVB and no difference between the two treatment regimens, indicating that phototherapy or photochemotherapy should certainly still be considered for patients with higher skin phototypes (45).

Given that three-times weekly NB-UVB results in faster more efficient clearance of psoriasis than twice-weekly treatment (46), comparison of twice weekly PUVA with a twice-weekly NB-UVB regimen is likely to be including a sub-optimal NB-UVB treatment arm. Indeed, in an intra-individual randomized controlled study of three times weekly NB-UVB with twice-weekly TMP bath PUVA, NB-UVB was of superior efficacy and also resulted in more rapid response of psoriasis, with 75% clearance compared with 54% with PUVA (40). Additionally, in a randomized intra-individual half-side study in patients with chronic plaque psoriasis, comparing three times weekly TMP bath PUVA and three times weekly NB-UVB, again NB-UVB was of superior efficacy compared with TMP bath PUVA, although all patients relapsed within 4 months of follow-up (47). In contrast, Salem et al., undertook a randomized controlled trial in 34 patients, comparing 8-MOP bath PUVA three times a week with three times weekly NB-UVB and greater reduction in PASI score was seen with PUVA than NB-UVB, along with greater reduction in peripheral CD4+ T Cells, indicative of possible systemic effects (48). Furthermore, Markham et al., undertook an open randomized inter-individual comparative study of twice-weekly oral 8-MOP PUVA with three times weekly NB-UVB for chronic plaque psoriasis and showed equivalent efficacy in terms of time to clearance and period of remission (49).

Thus, trying to make sensible conclusions from this diverse range of study findings, given the ease, convenience, and safety of treatment and the study evidence, NB-UVB should usually be considered as the first phototherapeutic option for patients with chronic plaque psoriasis, with PUVA used when NB-UVB is not effective or there is rapid relapse once NB-UVB is discontinued (39). A lower threshold for considering PUVA is reasonable if psoriasis is particularly thick and/or extensive at baseline, including erythrodermic and pustular psoriasis (50) or the patient is of higher skin phototype. In addition, 8-MOP bath or oral PUVA may be preferable to TMP bath PUVA, as although no head to head comparison has been undertaken, lower response rates are reported for those studies using TMP bath PUVA rather than 8-MOP (40, 47–49). Erythemogenic doses of PUVA are not a pre-requisite for clearance (51) and maintenance PUVA or NB-UVB for psoriasis should generally be avoided (52). Failure to respond to NB-UVB does not equate to prediction of a lack of response to PUVA and the latter should be considered for those who fail to do well with NB-UVB. For children, NB-UVB phototherapy is preferred and PUVA is relatively contraindicated, although this is not an absolute rule, but given the concerns about long-term safety, PUVA would not be the first line choice.

ECZEMA

Whilst any light-based treatment approach is less straightforward for eczema than psoriasis, not least for the reason of flaring of eczema in the early stages of treatment mainly due to the heat load of therapy, both NB-UVB and PUVA can be highly effective for the treatment of atopic eczema and other forms of eczema (5, 6). However, the evidence-base is relatively weak and there are no prospective studies comparing head-to-head systemic PUVA with NB-UVB (53). Systemic 5-MOP PUVA was shown to be superior to medium dose UVA1 for atopic eczema in an intra-individual randomized controlled comparison study (54). Bath PUVA can also be highly effective for atopic eczema (55). Bath PUVA using 8-MOP was compared with NB-UVB in a small half-side comparison study, showing that both were effective for severe atopic eczema without a significant difference between the two therapies (56). Thus, NB-UVB would usually be the first line of choice for atopic eczema, given the ease of administration, safety, and potential for use in children (57). Given the response of atopic eczema to several types of light-based therapy and if NB-UVB phototherapy fails or there is early relapse after discontinuation of treatment, then the options of either PUVA or UVA1 exist, although given the lack of evidence of superiority of UVA1, the latter would likely only be considered if PUVA was contraindicated. Indeed, a combination of NB-UVB and UVA or UVA1 could be considered for some patients, although whether this is advantageous compared with UVB alone is unclear and this needs further study (58).

VITILIGO

For the treatment of vitiligo, NB-UVB has been shown to be superior to PUVA with respect to rates of repigmentation, particularly for unstable extensive vitiligo, and in achieving more cosmetically acceptable even repigmentation (59–63). Thus,

NB-UVB would be the phototherapy of choice for vitiligo, although PUVA may be considered in certain cases, particularly if there is lack of response to NB-UVB.

CUTANEOUS T-CELL LYMPHOMA

Whilst there are no direct head-to-head controlled trials of NB-UVB and PUVA for early stage CTCL, both have been shown to be effective for this stage of disease (5, 64). In one retrospective study 81% of patients with early stage CTCL achieved complete remission with NB-UVB, compared with 71% with PUVA ($n = 56$) (65). This observation has also been supported by two other studies showing equivalent efficacy for NB-UVB and PUVA in achieving remission of early stage CTCL (66, 67) and thus NB-UVB should be the phototherapy of choice for early patch stage CTCL disease, with complete remission in approximately three quarters of patients being achievable, although duration of remission has not been thoroughly evaluated and relapse may occur within 6 months (68). It is unclear whether phototherapy has any impact on limiting natural disease progression. Based on one study it was suggested that tumor stage CTCL was slower to develop and overall survival was improved in those who had previously received phototherapy, although given the retrospective nature of the study these data must not be obver-interpreted (69). For thicker plaque stage CTCL, the increased depth of penetration of PUVA is desirable and NB-UVB would not be indicated, whereas PUVA would be the phototherapeutic modality of choice (5). For tumor stage disease, PUVA as monotherapy would not suffice and combination therapy is likely to be required. Maintenance PUVA should generally be avoided, but occasionally is justified for maintenance use in CTCL (5, 70). However, other adjunctive agents should be considered and combination with retinoids, rexinoids, or interferon may be required or the use of radiotherapy for localized tumor stage disease or total skin electron beam treatment for more extensive involvement (5). Photopheresis may of course be required for Sezary syndrome (71, 72). Thus, in summary NB-UVB for early stage disease and PUVA for plaque stage disease as monotherapy or in combination therapy for more advanced disease should be considered as mainstays in management (5, 64, 73)

THE PHOTODERMATOSES

There is a relative lack of randomized controlled trial evidence investigating the use of NB-UVB and PUVA for the abnormal photosensitivity conditions. However, for desensitization of PLE, comparative studies show equivalent efficacy for NB-UVB and PUVA (16). As regular annual desensitization courses may be required from a relatively young age, NB-UVB is preferred for PLE as the phototherapy of choice, although PUVA should be considered for treatment failures and when reported its use may be for more severe PLE (74, 75). Induction of PLE during treatment is common and to be expected but does not usually require early termination of the desensitization course and can usually be accommodated with reduction of dose increments and topical corticosteroid use during the treatment course (16, 76). With the other less common photodermatoses, desensitization phototherapies with either NB-UVB or PUVA may be considered and appropriate but will depend on the action spectrum for induction of abnormal photosensitivity and thus which light-based treatment approach can be tolerated. In general, these patients should be investigated and managed through a specialist photodermatology unit as there may be additional needs, such as inpatient requirements for suppression and light-protected care and advice regarding subsequent natural sunlight top up exposure. In CAD, the action spectrum for induction of abnormal photosensitivity is usually maximal in the UVB region and therefore NB-UVB phototherapy cannot often be tolerated. In this setting PUVA may need to be considered, sometimes in combination with topical superpotent or systemic corticosteroids in order to reduce the risk of disease flare, particularly in the early stages of treatment (77, 78).

NB-UVB and PUVA may also be useful therapeutic approaches for the other photodermatoses, such as erythropoietic protoporphyria, hydroa vacciniforme, actinic prurigo, and idiopathic solar urticaria (79). Indeed, in solar urticaria the action spectrum for induction of urticaria is usually in the UVA and visible parts of the spectrum and NB-UVB responses are typically normal, in which case NB-UVB desensitization can be used successfully for desensitization, with UVA rush hardening and/or PUVA considered if NB-UVB is not feasible or successful (79–84).

It would generally also be advisable for patients with solar urticaria to have anti-histamine cover whilst receiving a UV-based therapy. In EPP, as photosensitivity is maximal in the visible part of the spectrum, NB-UVB is usually well-tolerated and can be highly effective and is the phototherapy of choice. Whilst here is limited evidence to support the use of PUVA, given that patients with EPP will usually require annual treatment courses from a young age, NB-UVB is advised and PUVA is rarely justified (85–88). Similarly, whilst there is limited evidence to support the use of NB-UVB and PUVA in actinic prurigo, again given the young age and need for annual treatment, NB-UVB is advised and PUVA rarely needed, although may occasionally be required (79). Factors such as the age of the patient, risk factors such as skin phototype and evidence of photodamage and the action spectrum for induction of abnormal photosensitivity, should always be taken into account in any decision regarding NB-UVB or PUVA and for the photodermatoses, specialist advice regarding timing of desensitization courses, risk of induction of the condition by treatment and management of that, top-up exposure requirements after treatment and the need for annual treatment courses must be addressed in order to establish the optimal approach for any given patient.

LOCALIZED HAND AND FOOT DISEASE

Hand and foot dermatoses are a mixed group of conditions, which include hyperkeratotic eczema, psoriasis, psoriasiform dermatitis, palmoplantar pustulosis. There is a lack of robust evidence regarding the optimal management of these diseases, including the role of NB-UVB and PUVA therapies and there is

no reason to consider that one approach will suit all conditions. Undoubtedly, NB-UVB and PUVA photochemotherapy may be useful for localized hand and foot dermatoses (89). Although oral PUVA and NB-UVB may both be effective for eczema of the palms and soles, oral PUVA has been shown to be superior to NB-UVB in two small studies from the same group, although relapse rates were high following both treatments (5, 90, 91). The depth of penetration of 8-MOP systemic PUVA may be desirable for recalcitrant hand and foot dermatitis and other uncontrolled studies have also shown high levels of efficacy with oral PUVA for hand and foot eczema (5, 92, 93). In contrast, topical PUVA has not been shown to be superior to placebo or any other active treatment, despite uncontrolled studies, and anecdotal observations that efficacy can be achieved and this is an area requiring further research. Thus, for hand and foot eczema, oral PUVA would be the light-based therapy of choice (5). Psoriasis of the palms and soles has been even less well evaluated and, whilst there is some evidence to support the use of PUVA, either with oral or topical psoralens, the strength of evidence is weak and further studies are required (5, 7, 94). For palmoplantar pustulosis, again oral PUVA either as monotherapy or combined with retinoids, may be highly effective (5, 95–97) and the role of NB-UV is less clear as has not been evaluated.

OTHER INDICATIONS

There is evidence that NB-UVB and PUVA may be effective for urticaria and indeed randomized controlled trial evidence to show the superior efficacy of NB-UVB plus anti-histamine compared with anti-histamine alone (98–100). More recently, superiority of NB-UVB compared with PUVA has been shown for urticaria (101), and thus NB-UVB should be considered as a treatment option if antihistamines and other pharmacological therapies fail and may provide useful disease remission. A range of other conditions may be effectively treated by NB-UVB and PUVA and include pityriasis lichenoides (102), granuloma annulare (103, 104), urticaria pigmentosa and cutaneous mastocytoses (105–107), aquagenic pruritus (108–110), lichen planus (111–114), alopecia areata (115–118), generalized pruritus, such as secondary to uraemia or cholestasis (119, 120), and graft vs. host disease (2, 3, 5, 6) and these phototherapeutic modalities may be invaluable treatment approaches for these otherwise difficult-to-treat groups of diseases. For conditions such as pityriasis rubra pilaris, which may be aggravated and flared by the use of NB-UVB, 8-MOP systemic PUVA should be considered.

CONCLUSIONS

To summarize, NB-UVB phototherapy and PUVA photochemotherapy are both invaluable treatments to have available in any dermatology department and should be prioritized, not only for psoriasis, but in a variety of other inflammatory and proliferative skin diseases, including atopic eczema. Treatment can be safely and easily administered and is well tolerated with few adverse effects. Excellent disease remission may be achieved, whilst sparing the use of other potentially toxic drugs at a relatively early stage in a patient's journey. Head-to-head comparative monotherapy studies with biologic therapies do not exist and are needed. Due to the relative cost-efficacy of the phototherapies and the understanding of their long-term safety profiles compared with the cost and less lengthy follow-up for the biologics, these should be employed prior to consideration of biologic treatments (1). As with any therapy, standardization of optimized treatment regimens, careful observation of treatments delivered and therapeutic outcomes, adverse effects and long-term follow-up studies, including determining any skin cancer risk, are essential. The development of the National Managed Clinical Network for Phototherapy has had a major impact on standardization, safety, and vigilance in delivery of our phototherapy practices in Scotland and has proved to be an invaluable tool, enabling the place of NB-UVB, and PUVA therapies to continue to be well-established in the treatment of skin disease.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

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REVIEW
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Ultraviolet A1 Phototherapy for Fibrosing Conditions

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In this article we describe efficacy and safety aspects of ultraviolet A1 (UV-A1) phototherapy in fibrosing conditions. UV-A1 is a specific phototherapeutic modality that is defined by a selective spectral range (340–400 nm). UV-A1 includes distinct modes of action qualifying this method for therapy of a variety of conditions, in particular fibrosing skin diseases. Concerning efficacy of UV-A1 phototherapy in fibrosing conditions, the best evidence obtained from randomized controlled trials exists for localized scleroderma. Moreover, fibrosing disorders such as lichen sclerosus and graft-vs.-host disease can be treated successfully by means of UV- A1. Regarding the optimal dosage regimen medium-dose UV-A1 seems to be linked to the best benefit/risk ratio. Possible acute adverse events of UV-A1 phototherapy include erythema and provocation of photodermatoses. Skin ageing and skin cancer formation belong to the chronic adverse events that may occur after long-term UV-A1 phototherapy.

Keywords: UV-A1, ultraviolet A1, UVA1, irradiation, sclerosis, fibrosis, phototherapy

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INTRODUCTION

In order to reduce adverse effects such as erythema UV-A1 (340–400 nm) light sources were previously developed by eliminating the UV-A2 wavelengths (320–340 nm) which range to the UV-B (280–320 nm) spectrum (1, 2). Compared to UV-B and UV-A2, UV-A1 is thus less erythematogenic and does penetrate deeper into the skin (3). UV-A1 is a beneficial phototherapeutic modality for the treatment of disorders including eczema, urticaria pigmentosa, cutaneous T cell lymphoma, and in particular, fibrosing skin diseases (4–14). The present review focuses only on the fibrosing skin diseases and, although UVA1 may be beneficial in other conditions, they are not the focus of this review. We will also summarize the evidence in table format for each of the diseases discussed in the following review.

UV-A1 Light Sources and Regimens

UV-A1 Devices

Fluorescent bulbs (i.e., TL10R 100W, Philips, Eindhoven, Netherlands) and high-output metal halide lamps (i.e., Sellamed 4,000W, Sellas Medizinische Geräte GmbH, Ennepetal, Germany) belong to the commercially available UV-A1 sources. For practical reasons, fluorescent lamp cubicles are rather used for low to medium-dose UV-A1 phototherapy. By contrast, high-output metal halide lamps can also be used for high-dose UV-A1 since they deliver doses up to 130 J/cm^2 in acceptable time per treatment session. UV-A1 light sources designed for phototherapy have to fulfill some technical requirements. Hence, the amount of wavelengths smaller than 340 nm must be smaller than five percent of the total erythema-effective fluence. Furthermore, wavelengths smaller 320 nm as well as infrared should also be widely filtered out. Thus, irradiance of wavelengths between 800 nm and 1 mm must not be greater than five percent of the total fluence

(15). Fluorescent lamp whole- body devices are relatively inexpensive but have considerably lower spectral output as compared to metal halide devices (15). Nevertheless, duration of irradiation using high-output UV-A1 beds may also be long as the patient must usually treat subsequently to two body sides (15). Using UV-A1 metal halide lamps exposure times of thirty to sixty minutes per session are not uncommon, of course depending on fluence, indication and dosage regimen (14, 16).

Dosage Regimens

In order to be consistent with previous publications that are discussed in the present review we use the dosage categories as follows: low-dose UV-A1 (10–20 J/cm^2), medium-dose UV-A1 (>20–70 J/cm^2), and high-dose UV-A1 (>70–130 J/cm^2). Before starting UV-A1 phototherapy the medical history (i.e., photo skin-type, sun sensitivity, skin cancer) of the patient has to be checked also including the use of photo-allergic medications and immune-mediated photodermatoses. Importantly, immunosuppressants, including azathioprine, must not be combined with UV-A1 (17).

Given that there may be considerable variability in individual susceptibility to UVA1 erythema, undertaking an MED prior to starting treatment is preferred where feasible. If this proves not to be the case then a fixed start dose, for example, 20 J/cm^2 would usually be a safe approach, but there would then be the concern of potential under-treatment if running at doses that are well below the erythemal threshold (18, 19). UV-A1-MED data of two recent studies indicate that 20 J/cm^2 do usually not lead to erythema (20–22). Regular UV-A1 dosimetry is highly recommendable. The irradiance of the light sources should be assessed at different test sites whereby the mean value of all measurements defines the irradiance used for dose calculations (23).

Localized Scleroderma

High-dose UV-A1 therapy of localized scleroderma (LoS) was first reported by German researchers in 1997 (24). Stege et al. compared 10 patients receiving high-dose UV-A1 therapy with seven patients who were exposed to low- dose UV-A1 therapy. Stege et al. showed that UV-A1 significantly increased skin elasticity and decreased thickness and stiffness of the skin–these effects were particularly seen following high-dose UV-A1 (24). The latter findings are supported by *in vitro* analyses showing UV-A1 to reduce cell proliferation and dose-dependently decrease of collagen and hydroxyproline levels.

Moreover, a mouse model of scleroderma showed for high-dose UV-A1 a marked therapeutic effect on scleroderma. An improvement of dermal sclerosis and softened skin tissue could be observed (25). These results are in line with another mouse model study by Karpec and colleagues who investigated in scleroderma patients the impact of high-dose UV-A1 on dermal sclerosis. They could demonstrate that a total dose of 1,200 J/cm^2 does obviously not only prevent worsening of dermal fibrosis but also leads to a decrease of fibrotic skin changes (26). A further study by this working group showed in an animal model employing bleomycin- induced scleroderma that UV-A1 (cumulative doses: 1,200 J/cm^2 and 600 J/cm^2) is effective as well safe in the management of scleroderma (27).

By contrast, there is a wealth of data confirming the efficacy of low-dose UV-A1 therapy. Kerscher et al. (28) reported for the first time on a successful low-dose UV-A1 treatment in LoS patients ($n = 10$). Later, they conducted a study including 20 LoS patients who were treated with low-dose UV-A1 over 12 weeks. UV-A1 resulted in remarkable clinical improvement in 80% of the patients (29). However, patients ($n = 2$) with subcutaneous LoS did not respond to treatment. In a small study performed by Gruss and co-workers, the results mentioned above were supported as well (29). Moreover, LoS patients were treated three times per week using UV-A1 phototherapy (30 J/cm^2, treatment duration 10 weeks) (30). In all patients, softening of skin lesions was reported by the authors (30).

de Rie et al. reported on a controlled medium-dose UV-A1 trial including eight patients suffering from LoS (31). UV-A1 was given four times weekly over three months resulting in a decrease of skin fibrosis (cumulative dose: of 2,304 J/cm^2 UV-A1). We previously performed a comparative trial investigating low- dose UV-A1 (20 J/cm2), medium-dose UV-A1 (50 J/cm2), and narrowband UV-B for patients with LoS (32). Sixty-four patients suffering from LoS were treated in a randomized controlled trial including three treatment arms (15). Severity of LoS was evaluated using a simple clinical score. Phototherapy was performed five times weekly over two months. Kreuter (32) observed a significant improvement of LoS in all patients who completed the study which was shown by a decrease of clinical symptoms in all study arms assessed (15, 32). However, medium-dose UV-A1 was significantly more effective than narrowband UV-B (32). While low-dose and medium-dose UV-A1 were equally beneficial, substantial differences between low-dose UV-A1 and narrowband UV-B and medium-dose UV-A1 could not be observed.

Sator et al. (33) treated three clinically comparable LoS plaques in sixteen patients using 20 J/cm^2 UV-A1, 70 J/cm^2 UV-A1, or non-irradiation (32). Thirty therapy sessions were applied in total. Sator et al. (33) assessed thickness of the skin using high-frequency sonography and clinical score. Sonography revealed a significantly greater decrease of skin thickness for medium-dose UV-A1 when compared to low-dose regimen. By contrast, clinical scoring of fibrotic lesions irradiated also decreased markedly but did not show a clinically meaningful difference between medium-dose and low-dose UV-A1 (32). Together, the authors found that medium-dose UV-A1 for LoS resulted in more favorable long-term results when compared to low-dose UV-A1 as confirmed by sonographic assessments. High-frequency sonography is likely a more sensitive tool for the assessment of UV-A1-induced skin changes in LoS patients (33).

A recent cohort study by Vasquez and colleagues investigated recurrence risk of morphea after successful UV-A1 therapy–they observed the duration of LoS prior to therapy as the only associated variable. There was no difference in recurrence risk between different subtypes of morphea, skin types, adults and children, and medium to high dose regimens. Thus, the authors conclude that treatment doses in the medium- and high-dose UV-A1 range are adequate regarding the frequency of recurrence

(34). Su et al. (35) treated 35 LoS patients with medium-dose UV-A1 (30 J/cm^2). Medium-dose UV-A1 therapy improved fibrotic lesions in all patients. A substantial treatment success was found in 29 of 35 patients. Ultrasound measurements demonstrated that the thickness of skin significantly decreased after medium-dose UV-A1. There were no detectable treatment related adverse events.

Moreover, Andres et al. (36) demonstrated in LoS patients a favorable short-and long-term effect through medium-dose UV-A1 therapy, including diminishment of fibrotic lesions, improvement of skin elasticity, and decrease of skin thickness. Furthermore, Pereira et al. (37) conducted a retrospective evaluation of LoS patients who had underwent low-dose UV-A1 (average dose: 31 J/cm^2) phototherapy (32). They treated 18 patients with LoS showing a substantial improvement in more than three-fourth of patients and a modest improvement in 12% of patients (37). Moreover, Gruss et al. (38) reported on disabling pansclerotic morphea of childhood who was successfully treated with low-dose UV-A1 (cumulative dose: 640 J/cm^2 UV-A1) four times weekly over two months resulting in substantial reduction of skin fibrosis.

Together, medium UV-A1, based on the evidence base would be considered as the phototherapeutic treatment of choice for patients with LoS (**Table 1**). However, it is worth emphasizing that there is no head-to-head comparison between UV-A1 and psoralen plus UV-A (PUVA) for scleroderma, and this would be an important study with regards to establishing the place of UVA1 in the phototherapeutic approaches of scleroderma, as at present we do not know whether UVA1 is equivalent, inferior or superior to PUVA.

Systemic Sclerosis

von Kobyletzki et al. (40) reported on eight patients suffering from systemic sclerosis (SSc) whose acrosclerosis was treated with low-dose UV-A1. They used 30 J/cm^2 UV-A1 four times per week over two months and thereafter three times weekly over a six week period (50 treatment sessions in total, cumulative dose: 1,500 J/cm^2) (40). Morita et al. (41) also observed UV-A1-induced softening of skin fibrosis (cumulative dose: 510 to 1,740 J/cm^2) in four patients with SSc. In another paper they also found UV-A1-induced decrease of dermal decorin expression in SSc patients (41, 42). In an open non-randomized study we previously treated 18 patients with acrosclerosis and underlying SSc. Applying the UV-A1 regimen described by von Kobyletzki et al. (40), Kreuter et al. (43) observed skin softening, enhancement of skin distension, decrease of thickness of skin, and increase of cutaneous collagenase activity in 16 of 18 patients (32).

Pereira et al. (37) reported three SSc patients who were treated with medium-dose UV-A1. In two patients, acrosclerosis improved significantly (37). Moreover, Rose et al. reported on eight SSc patients (diffuse type, $n = 5$; limited type, $n = 3$) who showed skin fibrosis predominantly on acral and proximal extremity sites. The patients were treated using UV-A1 (30–40 J/cm^2) 3 times per week. Skin fibrosis improved as indicated by a decrease of the modified Rodnan skin score (32). Hence, this study also demonstrated that UV-A1 treatment is effective in SSc patients, particularly for acrosclerosis (44). In contrast, Durand et al. (45) reported a randomized observer-blinded half-side controlled trial on UV-A1 treatment of acrosclerosis. They used low-dose UV-A1 (40 J/cm^2) three times per week (14 weeks treatment period). Although a marked improvement of the clinical scores was observed, no difference could be detected regarding the clinical outcome of irradiated and non-irradiated extremities (32).

In contrast to the aforementioned results, the data of Durand et al. (45), which was based on a controlled investigation, suggest that UV-A1 therapy is ineffective in acrosclerosis (45). Otherwise one must consider a systemic UV-A1 effect that could explain the results of Durand et al. (45). Moreover, Tewari et al. reported medium-dose UV-A1-induced reduction of microstomia in a SSc patient (46). Jacobe et al. (9) effectively treated 34 SSc patients. On the basis of their data, medium- to high-dose UV-A1 therapy seems to be similarly effective independently of patients photo-skin types. Nevertheless, outcome measures were not reported in detail (9). In another study on 16 SSc patients, a statistically significant dose-response association was found between low-, medium-, and high-dose treatment regimens (47). Notably, Comte et al. reported UV-A1- induced improvement of Raynaud's phenomenon observed in over 80% of patients ($n = 11$) with autoimmune disorders including SS (48).

In contrast to the well-documented evidence of beneficial UV-A1 efficacy in LoS the data for SSc are pretty contradictory and of much poorer quality. Hence, UV-A1 should not be considered a first-line treatment modality for SSc patients.

Lichen Sclerosus

In a prospective non-controlled study, we treated ten patients suffering from extragenital lichen sclerosus (LiS) with low-dose UV-A1 (20 J/cm^2) therapy 4 times weekly (32). After low-dose UV-A1 therapy a remarkable decrease of the clinical score and normalization of skin texture was observed as also confirmed

TABLE 1 | UV-A1 treatment for fibrosing conditions—levels of evidence as proposed by the American College of Cardiology and the American Heart Association (39).

Levels of evidence	Indications/protocol
Level A	
Data derived from multiple randomized clinical trials or Meta-analyses	Localized scleroderma §Medium-dose 60 J/cm^2 3–5 times weekly total of 40 sessions
Level B	
Data derived from a single randomized trial, non-randomized studies, prospective case studies	Lichen sclerosus §Medium-dose 50 J/cm^2 5 times per week total of 40 sessions
Level C	
Only consensus opinion of experts, retrospective case studies, case reports, or standard-of-care	Systemic sclerosis* Nephrogenic systemic fibrosis GvHD

**conflicting data, §medium-dose UVA1 >20–70 J/cm.*

by sonography. The patients noticed substantial skin softening and repigmentation in pre-existing lesions. It was suggested that similar to therapy outcomes in LoS, low- dose UV-A1 therapy seems to be a beneficial and well-tolerated therapy modality for extragenital LiS (32). Rombold et al. (11) also observed beneficial outcome for LiS patients managed with medium-dose UV-A1 (cumulative dose: 1,018 ± 575.3 J/cm^2).

Beattie et al. (49) evaluated the efficacy of UV-A1 in genital LiS. Seven females were exposed to UV-A1 (low- to high-dose protocol according to MED). Five patients responded to treatment, three patients showed modest clinical improvement, and two experienced only slight therapy success. Of the five responders, one had disease relapse within three months and another after one year. The latter patients were re-treated by means of UV-A1 therapy – one had minimal improvement, the other had moderate treatment success. In the other responders, the condition substantially improved and was controllable using topical glucocorticosteroids. The authors suggested that UV-A1 is potentially an effective treatment approach for genital LiS, particularly considering that this disease is frequently poorly manageable (49).

Data of a randomized controlled trial performed in our department comparing the efficacy of high-potent topical glucocorticosteroids (clobetasol propionate 0.05%) with UV-A1 therapy (50 J/cm^2, 4 times per week over 12 weeks) in the management of 30 patients with genital LiS showed a significant improvement of symptoms. Nevertheless, the current gold standard, say high-potent glucocorticosteroids, was superior to UV-A1, particularly with respect to practical considerations, reduction of pruritus, and quality of life improvement. However, we suggested to consider UV-A1 phototherapy as potential second-line treatment for VLiS (50). Moreover, our study group investigated epigenetic changes in 10 patients with LiS before and after a medium-dose UV-A1 (up to 50 J/cm^2, 4 times weekly for 3 month) treatment compared to healthy controls. It could be shown that UV-A1 phototherapy may cause a normalization of 5-hydroxymethylcytosine levels–epigenetic factors may also contribute to LiS pathophysiology (15, 51).

Conclusively, based on data derived from a single randomized trial, non-randomized studies, and prospective case studies UV-A1 appears to be a treatment option for genital and extragenital forms of LiS.

Graft-vs.-Host Disease

Previously, Grundmann-Kollmann et al. (52) reported a patient suffering from chronic sclerodermic graft-vs.-host disease (GvHD) who was refractory to conventional therapies (32). In the combination with oral mycophenolate mofetil low-dose UV-A1 (20 J/cm^2) four times weekly was beneficial (cumulative dose: 480 J/cm^2 UV-A1). Furthermore, Stander et al. (53) studied five GvHD patients receiving 50 J/cm^2 UV-A1 (5 times per week) over eight weeks followed by subsequent diminishment of UV-A1 doses toward 3 times weekly (32). Notably, one patient was irradiated using a fix dose of 20 J/cm^2 UV-A1 combined with immunosuppressants and extracorporeal photopheresis (ECP). In all patients, treatment resulted in skin softening of pre-existing lesions (53). Calzavara-Pinton et al. (54) treated five patients with sclerodermoid GvHD (localized: 4; generalized: 1) with medium-dose UV-A1 (50 J/cm^2) therapy three times weekly. Therapy was successful with complete responses observed in three patients and partial responses in two (54).

In contrast, a study of 25 GvHD patients by Connolly et al. found clinical improvement in patients who received high-dose UV-A1 phototherapy (47). In a small trial, 7 patients were exposed to UV-A1 as primary treatment for acute cutaneous GvHD. In 5 patients, a complete response was noticed, in 2 patients were non-responders and requiring systemic steroids (32). In 2010, Schlaak et al. (55) studied 70 patients suffering from acute cutaneous GvHD. Following a median therapy period of 10 months, the authors achieved complete and partial responses in 70% and 24.3% of patients, respectively. Following a median follow-up of 18 (range 10–60) months, non-melanoma skin cancer occurred in three patients. The authors concluded that UV-A1 therapy can be a beneficial therapy for acute GvHD affecting the skin (32). Avoiding chronic use of systemic glucocorticosteroids and/or allowing a faster tapering of immunosuppressants in a substantial number of patients, UV-A1 appears to be an interesting therapy option for GvHD (55). Moreover, Ziemer et al. treated two children with chronic cutaneous GvHD who improved after UV- A1 therapy with regard to cutaneous lesions, joint mobility, and quality of life (32).

The benefit of UV-A1 for GvHD patients has only been documented in small retrospective case series and case reports making it difficult to give a definitive recommendation for this photherapeutic modality in GvHD. Moreover, there are no comparison studies with UV-A1 and ECP–a frequently recommended photochemotherapeutic option for GvHD patients.

Nephrogenic Systemic Fibrosis

Tran et al. (56) recently treated nephrogenic systemic fibrosis (NSF) with UV-A1 phototherapy. All patients ($n = 4$) received hemodialysis before, during, and after high-dose UV-A1 (32). All patients noticed softening of their skin, and two patients experienced increase of mobility of the limbs. The therapeutic was significant in all cases, even though none patient complete clearance of fibrosis could be achieved. Hence, UV-A1 represents a feasible therapy modality for NSF, in particular in cases in which kidney transplantation is no option or in delay (56). Interestingly, UV-A1 does not only improve clinically NSF but also induce procollagen synthesis and reduce profibrotic cytokine and growth factor expression (32). Using a medium-dose regimen, however, we could not observe beneficial effects after UV-A1 therapy in patients ($n = 3$) with NSF (32). These results are supported by an analysis of 17 patients with NSF which found high-dose regimens to be more effective than medium- and low-dose regimens for NSF (47). By the way, Gazi et al. (57) performed a survey, and found that an reduction of 3 to 7.5 points of the modified Rodnan skin score does reflect a clinically meaningful treatment outcome (32).

In conclusion, UV-A1 may work in NSF; however, this statement is only based on a few case series and retrospective observations.

Miscellaneous

Moreover, positive results following UV-A1 phototherapy of fibrosing conditions have been documented in case reports on patients with scleromyxedema, scleredema adultorum Buschke, and pansclerotic porphyria tarda. Variable data have been reported for UV-A1 therapy of keloids and eosinophilic fasciitis (5, 7, 11, 58–63).

Mechanisms of Action, Limitations, and Adverse Events

Photo-Skin Type Status

It is still controversially discussed whether patients with photo-skin type > III respond worse to UV-A1 therapy (7, 45, 64). Wang et al. (64) demonstrated that a single UV-A1 dose can markedly reduce procollagen mRNA gene expression and substantially enhance matrix metalloproteinase 1 and 3 gene expression in controls (15). Their results showed that such anti-fibrotic effects likely decrease after repeated UV-A1 irradiation sessions (15). By contrast, skin darkening usually depends on dosage (15). Stronger pigmentation resulted in a decrease of the anti-fibrotic effects of UV-A1 (15). Hence, individuals with dark skin show only marginal or even no decrease of procollagen when compared to individuals with fair skin (15). The aforementioned results could have significant implications on patient stratification for therapy, proposing that patients with fair skin are better candidates for UV-A1 therapy (15). Wang et al. (64) speculated that the aforementioned observation may be the reason for more favorable outcomes reported in previous UV-A1 trials on sclerotic skin diseases predominantly including European Caucasians (15).

Tuchinda et al. (7) reported ($n = 92$) that patients with fair skin likely respond to UV-A1 better than patients with darker skin (15). However, Jacobe et al. (9) performed a study on 101 patients who were treated with UV-A1 treatment. Photo-skin types and total UV-A1 doses were analyzed. The evaluation of therapy outcome was based on clinical parameters such body surface area, fibrosis and subjective symptoms such as itch (15). Interestingly, clinical response to UV-A1 was not dependent on skin complexion in this population assessed.

Mode of Action Aspects

More infrequent types of LoS including linear LoS an deep morphea and severe cases of acrosclerosis and sGVHD frequently affect deeper anatomical structures such as fascias, muscles, and bones (15). Because UV-A1 penetrates into the subcutis only, the aforementioned conditions rather require systemic immunosuppressive treatment such as methotrexate (65). Evidence indicates that UV-A1 phototherapy acts through diminishment of cutaneous T cell infiltrates, down-regulation of pro-inflammatory cytokines, changes in endothelial cell function, and induction of programmed cell death. Nevertheless, the most important mode of action of UV-A1 in fibrotic conditions is the induction of matrix metalloproteinases and inhibition of collagen synthesis (15). Furthermore, UV-A1 exerts changes in fibroblast cytokine production (15) such as transforming growth factor- ß/Smad signaling and interleukin (IL) and IL-6, leading to an upregulation of collagenase activity (15). It was shown *in vitro* that UV-A1 irradiation of cultured fibroblasts obtained from LoS patients resulted in increased collagenase gene and protein expression. After UV-A1 irradiation, it was also observed a fast production of interleukin 1 (IL-1) stimulating the release of IL-6 which mediates an upregulation of collagenase synthesis by fibroblasts (14, 65–71).

Side Effects

The most common acute adverse events of UV-A1 include increased pigmentation, erythema, and itch (5, 10, 12, 14, 72). UV-A1 treatment usually needs long exposure times, resulting in considerable heat, which might be intolerable for patients. Phototoxic reactions may occur, in particular in patients with fair skin (20). Notably, UV-A1 absorbing substances of the skin, such as porphyrins and riboflavins, can cause oxidative stress resulting in phototoxic reactions (73). Beside the aforementioned side effects, Wang et al. (74) investigated the effects following a limited number of low-dose UV-A1 irradiation sessions as usually experienced in daily life. They observed that these UV-A1 exposures potentially promoted photoaging by affecting breakdown, rather than synthesis, of collagen. In fair skinned individuals, increasing skin pigmentation due to low-dose UV-A1 did not prevent collagenolytic alterations usually induced by UV-A1. They concluded that sunscreens must block sufficiently UV-A1 wavelengths as well (74). Furthermore, UV-A1 can induce photodermatoses or reactivate herpes flares (16, 75). A recent case study reported a 37-years-old female with a persistent polymorphous light eruption lasting for 5 weeks following UV-A1 phototherapy (76).

Skin cancer and premature skin aging belong to the most important chronic side effects linked to broadband UV-A radiation. UV-A can suppress skin immunity in a bell-shaped dose response (15). Long-wave UV-A corresponding to dose equivalents of 20 min sun exposure contributes to about 75% immunosuppression caused by sun irradiation (15). It was shown that UV-A1 but not UV-A ranging from 320 to 350 nm induces immunosuppression in humans, indicating a significant role for reactive oxygen species (77). Moreover, UV-A induces an energy crisis in cells, can activate alternative complement pathways, and alters the development of memory T cells (15). Skin cancers are associated with p53 and BRM mutations, which can be induced by UV-A1 as well (77–79).

Of importance is also research of Tewari et al. who recently reported a study indicating the induction of DNA dimers at the basal layer and in the upper dermis after UV-A1 exposure (80).

Principally, patients treated with UV-A1 must have regular skin checks and should avoid the use of sunbeds and/or additional sun exposure (15). UV-A1 contraindications may include conditions of UV sensitivity (i.e., xeroderma pigmentosum, porphyrias), use of UV sensitizing substances, history of skin cancers, radiotherapy, and chronic immunosuppresssion (15). For example, azathioprine leads to increased UV-A sensitivity and thus is a well-known photocarcinogen (81).

Overall Conclusion

The best evidence of efficacy for UV-A1 therapy exists in LoS. We consider medium UV-A1 the first-line modality for disseminated forms of this disease, in particular given the fact that there is a lack of effective standard treatments. The latter does also apply to LiS which is closely related to LoS. Hence, UV-A1 represents an attractive treatment option for widespread LiS as well. In the other conditions discussed above UV-A1 may represent an alternative treatment option. About 6 years ago, Kerr et al. (23) considered that UVA1 should only be available through specialist services until we have more evidence. With regard to efficacy of UV-A1 we think that this phototherapeutic option should be widely available in all dermatology centers. However, the price for high-output UV-A1 devices is still very high. Hence, we are afraid that UV-A will predominantly remain a more specialized unit tertiary service.

AUTHOR CONTRIBUTIONS

Both authors contributed to the literature search, data extraction, interpretation of results, and preparation of the manuscript. Manuscript approval was performed by both authors.

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The Antipruritic Effect of Phototherapy

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Phototherapy is widely used to treat inflammatory skin diseases such as psoriasis and atopic dermatitis. Repeated suberythemogenic doses of UV-light reduce inflammation in these diseases and ultimately may lead to a complete disappearance of cutaneous symptoms for weeks or months. Chronic pruritus is an important and highly distressing symptom of many of these inflammatory skin diseases. Interestingly, pruritus is also reduced or completely abolished by UV-treatment of psoriasis and atopic dermatitis, and sometimes reduction of pruritus is the first indication for skin improvement by phototherapy. The cutaneous nervous system is an integral part of skin anatomy, and free nerve endings of sensory cutaneous nerve fibers reach up into the epidermis getting in close contact with epidermal cells and mediators from epidermal cells released into the intercellular space. Stimulation of "pruriceptors" within this group of sensory nerve fibers generates a neuronal signal eventually transmitted via the dorsal root and the spinal cord to the brain, where it is recognized as "itch". UV-light may directly affect cutaneous sensory nerve fibers or, via the release of mediators from cells within the skin, indirectly modulate their function as well as the transmission of itch to the central nervous system inducing the clinically recognized antipruritic effect of phototherapy.

Keywords: Pruritus, itch, chronic prurigo, prurigo nodularis, phototherapy, UV-light, psoriasis, atopic dermatitis

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INTRODUCTION

It has long been recognized that "UV-responsive" skin diseases improve during summer months and worsen during winter, and exposure to natural sunlight, i.e., heliotherapy, is a common way of psoriasis patients to improve their skin lesions. Phototherapy has shown significant effects in these "UV-responsive" skin diseases and is widely used to treat inflammatory skin diseases such as psoriasis, atopic dermatitis (AD) as well as cutaneous T-cell lymphoma (CTCL), e.g., mycosis fungoides/Sezary-Syndrome (1–3). Chronic pruritus (i.e., pruritus lasting for 6 weeks or longer) is an important and highly distressing symptom of many of these inflammatory skin diseases and significantly impairs the quality of life in the affected patients. Repeated suberythemogenic doses of UV-light, as used in phototherapy, are capable of reducing inflammation in these diseases and ultimately may lead to a complete disappearance of cutaneous symptoms for weeks or months. However, not only the skin lesions of these diseases improve but also the accompanying pruritus decreases when patients undergo repeated UV-treatments. Interestingly, phototherapy is capable of improving chronic pruritus in a variety of different pruritic skin diseases beside psoriasis and AD, such as lichen planus, pityriasis lichenoides, urticaria pigmentosa, chronic spontaneous urticaria, parapsoriasis, and CTCL (e.g., Sezary-Syndrome) (4).

Phototherapy, in addition, is also effective against chronic pruritus in systemic diseases such as end-stage renal disease, cholestatic liver disease (e.g., primary biliary cholangitis or cholestatic pruritus of pregnancy), hematologic diseases (e.g., polycythemia vera or Hodgkins lymphoma) and other conditions of chronic pruritus without primary or secondary skin lesions (e.g., drug induced pruritus after hydroxyethyl starch) (4, 5). Even in the various forms of chronic prurigo (6), including the severe nodular and umbilicated ulcer types, as well as in chronic idiopathic pruritus mainly in elderly patients, phototherapy is very effective and sometimes the only treatment improving chronic pruritus (5, 7).

When looking at the broad antipruritic effect of phototherapy the question arises how phototherapy is capable of reducing pruritus in such a variety of inflammatory skin and systemic diseases with obviously very different pathophysiological backgrounds?.

It is clear, that the antipruritic effect of phototherapy has to depend on the ability of UV light to interfere with structures and mediators involved in the induction and perception of pruritus. However, at the moment, the pathophysiology of pruritus in the various skin and systemic diseases is not completely understood and there is even less knowledge about the mechanisms how phototherapy is capable of reducing pruritus in these diseases. In the following paragraphs we try to approach the question of the antipruritic effect of phototherapy by looking at some targets of UV light in the skin and possible UV-induced mediators which may contribute.

UV-TARGETS IN THE SKIN

When UV-light impinges on the skin it reaches the most superficial layers including the cell-rich epidermis as well as the underlying dermis. The longer the wavelength, the deeper UV-light penetrates into the skin. Thus, while the shorter wavelengths of UVB mainly exert their effects in the epidermis and upper papillary dermis, UVA may penetrate into deeper dermal layers. These superficial layers of the skin reached by UV are also the skin layers where pruritus can be perceived (8), and it is a well-known clinical finding, that removal of the superficial skin layers leaves the skin devoid of itch perception, while pain can still be recognized.

In the epidermis, resident cells such as keratinocytes, melanocytes, and Langerhans cells, as well as infiltrating cells such as lymphocytes and leukocytes, can be reached and affected by UV. The connective tissue of the upper dermis, beside fibroblasts and the cells of blood vessels, sweat glands and sebaceous glands, hosts an array of other cells such as lymphocytes, leukocytes, dermal dendritic cells, mast cells, and eosinophils, which are important players in inflammatory and immunological processes.

Within the most upper part of the dermis, just beneath the epidermis, a subepidermal plexus is formed by cutaneous sensory nerves from which nerve fibers perpendicularly grow into the epidermis. As these nerves penetrate the basement membrane they lose their myelin sheath, reach up to the granular layer and stratum corneum and extensively branch within the epidermis. Lying within the intercellular space of the epidermis, these sensory nerves get in close contact with resident keratinocytes, melanocytes and Langerhans cells, or infiltrating lymphocytes and leucocytes. Within this group of intraepidermal sensory nerve fibers (IENF), the pruriceptive sensory nerve fibers, i.e., histamine-sensitive, mechano-insensitive nerve fibers and histamine-insensitive, mechanoheat-sensitive, "polymodal" nerve fibers, can been found. They take up the pruritic signals from the periphery and transmit them via their cell bodies in the dorsal root ganglia (DRG) and their central projections to the spinal cord and further to the brain (8).

UV-light, thus, reaches and may directly or indirectly interact with the dense three-dimensional network of sensory nerves within the epidermis and upper dermis.

Both, the interaction with the cellular components as well as with the nerve structures in this skin compartment may convey the antipruritic effects of phototherapy (**Figure 1**).

CHRONIC PRURITUS AND PHOTOTHERAPY

Among the first, who looked into the antipruritic effects of phototherapy in the clinic were Barbara Gilchrest and colleagues. In uremic patients on hemodialysis suffering from chronic pruritus, they could show that repeated broadband (BB)-UVB twice weekly compared to time-matched UVA significantly reduced pruritus in 9 of 10 patients (9).In their studies, they also showed that half-body UVB treatments reduced pruritus not only on the irradiated body half but equally reduced pruritus also on the non-irradiated body-half (10). This indicates that the antipruritic effect of BB-UVB on uremic pruritus in hemodialysis patients is mediated by a systemic, yet unknown effect. In this study they also found that the antipruritic effect is not immediate but requires several treatments and at least 2 weeks to being recognized by the patients. It also occurred that thrice weekly treatments accelerated the onset of the antipruritic effect compared to treatments only once a week, in which the antipruritic effect was not recognized before the 4th week.

In a clinical trial in patients with chronic pruritus with or without pruriginous skin lesions, some of them with renal insufficiency, the antipruritic effect of whole body narrowband (NB)-UVB was not inferior to broadband (BB)-UVB (11). Thus, NB-UVB, today the preferred treatment modality of phototherapy (12), is also effective in treating generalized chronic pruritus.

However, in other skin diseases associated with chronic pruritus such as AD, psoriasis, CTCL or pityriasis rosea, phototherapy with UVB or PUVA exerted a local effect on skin lesions and the associated pruritus (9). In a half-body study in patients with AD, treated with NB-UVB on one half and UVA-1 on the other half, patients were able to recognize differences in pruritus reduction by the two treatments indicating at least a partially local antipruritic effect of NB-UVB and UVA-1. However, an additional systemic effect of the two treatments cannot be excluded and is likely in a half-body study (13). A local

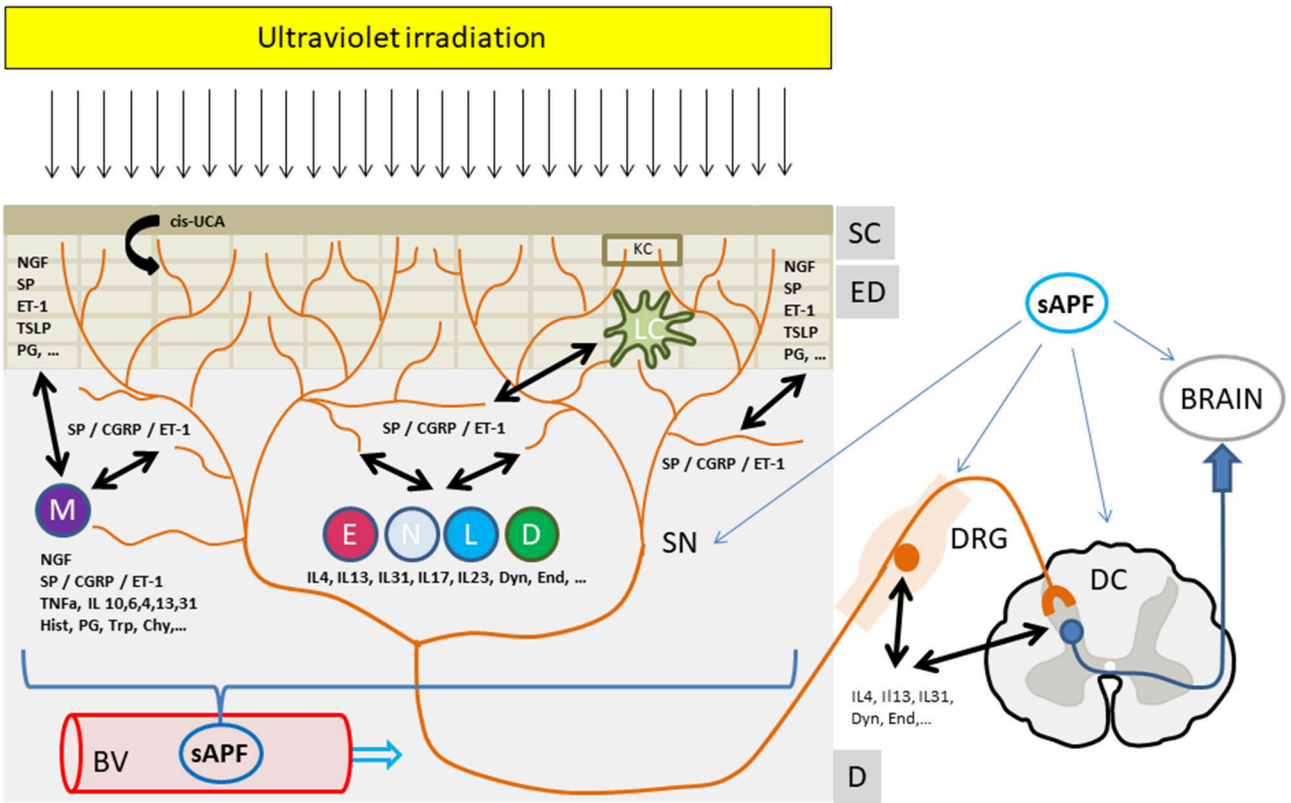

FIGURE 1 | The antipruritic effect of phototherapy. Ultraviolet irradiation reaches and affects all structures and cells within the upper skin layers from the stratum corneum to the epidermal and dermal layers. Upon UV irradiation multiple mediators from sensory nerves, resident or infiltrating cells are affected (decrease, increase, release). These mediators extensively interact with cutaneous nerves and cells eventually leading to an inhibition of itch perception and/or signaling to the brain. In addition, a yet unknown UV-induced "soluble anti-pruritic factor" (sAPF) from the skin may reach the peripheral as well as the central nervous system via the circulation and contribute to the inhibition of itch signaling and/or perception. See text for further details. **Mediators:** Cis-UCA, Cis-urocanic acid; ET-1, Endothelin-1; NGF, Nerve growth factor; CGRP, Calcitonin gene related peptide; SP, Substance P; IL, Interleukin; TNFa, Tumor necrosis factor alpha; Hist, Histamine; PG, Prostaglandins; Trp, Tryptase; Chy, Chymase; TSLP, Thymic stromal lymphopoetin; Dyn, Dynorphin, End, Endorphin; **Structures:** SC, Stratum Corneum; ED, Epidermis; D, Dermis; BV, Blood Vessel; DRG, Dorsal root ganglia; SN, Sensory nerve; DC, Dorsal column, **Cells:** KC, Keratinocyte; M, Mastcell; E, Eosinophil, N, Neutrophil; L, Lymphocyte, D, Dermal Dendritic cell; LC, Langerhans cell.

antipruritic effect can also be seen in targeted UV-treatments with UVB, UVA-1, or excimer laser (i.e., 308 nm), if single pruriginous nodules or circumscribed lichen simplex chronicus are treated (4).

Thus, it appears that the antipruritic effect of phototherapy involves both local as well as systemic factors, depending on the area of treated skin. This favors the idea of the induction of a soluble antipruritic factor by UVR eventually released into the circulation and affecting peripheral and/or central itch pathways (**Figure 1**). UV, however, may also locally affect the production and release of itch mediators as well as directly or indirectly change the sensitivity of cutaneous sensory nerves to itch signals. In any case, it has been recognized that only repeated suberythemogenic doses of UV-light induce the antipruritic effect of phototherapy while high doses of UV, especially in the UVB range, induces skin inflammation ("sunburn") and induces or aggravates pruritus. This implies that the antipruritic effect of phototherapy is also a matter of UV dose and treatment frequency, as shown by Gilchrest et al. (9) in uremic pruritus.

UV-EFFECTS ON THE OPIOID SYSTEM

The group of patients with end-stage renal disease, especially if undergoing hemodialysis, is especially prone to severe pruritus with up to 50% of hemodialysis patients being affected (14). Beside phototherapy with UVB, the systemic application of the μ-opioid receptor antagonists naloxone and naltrexone as well as the kappa-opioid receptor agonist nalfurafine have shown significant antipruritic effects (15). This implies that opioids are important mediators of uremic pruritus and may be among the soluble factors suggested to participate in the "systemic" antipruritic effects of phototherapy in uremic patients.

In addition, topical application of the μ-opioid antagonist naltrexone has shown antipruritic effects in patients with different chronic pruritic disorders (16). Topical application of the kappa-opioid-agonist nalfurafine also showed an antipruritic effect in a murine model of AD (17). Thus, opioids may play a role in both peripheral as well as central modulation of pruritus in uremic pruritus and other pruritic diseases such as AD, in

which decrease of kappa-opioid receptors (KOR) but not of μ-opioid receptors (MOR) have been found in the skin, resulting in a misbalance of the MOR over KOR system (18). In AD patients, PUVA has shown to decrease MOR not changing the level of its agonist β-endorphin, but increasing the KOR agonist dynorphin leaving the KOR expression unchanged. Together, these PUVA-induced changes resulted in a decreased activity of the "MOR system" together with an increased activity of the "KOR system," which correlated with a decreased VAS score for pruritus. The KOR agonist dynorphin is capable of modulating itch perception via e.g., interaction with KOR on interneurons in the spinal cord (19). Thus, an effect of UV on receptors and mediators of the opioid system may contribute to the antipruritic effect of phototherapy in ESRD, AD as well as in other pruritic conditions such as cholestatis, in which the MOR antagonists naloxone and naltrexone have also shown antipruritic efficacy and are recommended in the treatment for cholestasic pruritus (20). Phototherapy has also been reported to be effective in reducing cholestatic pruritus (21), and should be tried in case of resistance to guideline conform treatments.

UV-INDUCED IMMUNOSUPPRESSION AND THE CUTANEOUS NERVOUS SYSTEM

Systemic immunosuppressive agents such as methotrexate, azathioprine, or mycofenolate mofetil, and especially corticosteroids and cyclosporine, sometimes have shown remarkable antipruritic effects in various diseases such as AD, chronic prurigo, or Sezary-Syndrome, and they are still used in severe recalcitrant cases of chronic pruritus. The mechanisms by which immunosuppressive substances reduce pruritus in these various conditions, however, are not completely understood (22).

Phototherapy with repeated UV irradiations is also capable of inducing local as well as systemic immunosuppression. It is well-known, that the interaction of UV with the cellular components of the skin, mainly by interaction with DNA, leads to a sequence of events resulting in local and systemic immunosuppressive effects such as the suppression of contact hypersensitivity (CHS) and the induction of tolerance, in which T-regulatory cells play an important role (23).

It is less well-known, that the interaction of UV with the cutaneous sensory system also conveys local as well as systemic immunosuppressive effects. The same group of sensory nerve fibers within the epidermis and upper dermis, among which we find the pruriceptive nerve fibers, are also capable of mediating or modulating the immunosuppressive effects of UV.

In mice, acute and chronic UV radiation (UVR) is capable of inducing local and/or systemic immunosuppression (i.e., suppressing CHS). This UV-induced suppression of CHS was blocked in mice with impaired sensory nervous system by pretreatment of these mice with capsaicin on their 2nd day of life (24). Capsaicin is the pungent ingredient of hot chili pepper, which specifically targets capsaicin-sensitive C- and A-delta fibers, leaving rodents insensitive to further capsaicin challenges, if they have been treated with a high dose of capsaicin in the first days of live. In addition, pretreatment with a neuropeptide calcitonin gene-related peptide (CGRP) antagonist, CGRP 8–37, also abolished UV-induced suppression of CHS in mice (25). CGRP is an important neuropeptide within sensory nerve fibers and similarly to UVR is capable of reducing the number of Langerhans cells within the epidermis, which is important in mediating the local immunosuppressive effect of UVR (26). CGRP is often co-localized with substance P (SP), which is an important mediator of neurogenic inflammation via stimulation of neurokinin-1 receptors (NK1R). Both neuropeptides, SP and CGRP, are released by acute high dose UVR resulting in a neurogenic inflammation which contributes to the sunburn reaction (25). However, repeated low doses UVR of mice, increases SP- and CGRP-immunoreactive nerve fibers in the epidermis of irradiated skin compared to non-irradiated skin (27, 28). This increase in neuropeptides within sensory nerve fibers and the increase of the number of intraepidermal nerve fibers are most likely mediated by nerve growth factor (NGF) produced, e.g., by keratinocytes and mast cells upon UVR. NGF, after retrograde neuronal transport from the periphery to the DRG cells, increases the synthesis of neuropeptides and stimulates the outgrowth of sensory nerves in the skin (29). In peripheral inflammation, NGF is increasingly produced and can also induce the release of SP and CGRP from sensory nerve fibers (29).Via a feedback loop, SP acting on NK1R can again increase the production and release of NGF, e.g., by keratinocytes and mast cells. Thus, blocking the NK1R, also a target in antipruritic drug development (e.g., the NK1R antagonist serlopitant in chronic prurigo (30), reduces inflammation as well as NGF production, which may also affect UV-induced immunosuppression. Interestingly, systemic application of NGF is capable of suppressing CHS in mice, and this is abolished in mice with capsaicin-impaired neurosensory systems (31). Anti-NGF antibodies, on the other hand, similarly to the capsaicin-impairment of sensory nerves, are also capable of inhibiting UV-induced suppression of CHS, indicating that NGF and the cutaneous neurosensory system play significant roles in UV-induced immunosuppression.

Another factor mediating systemic immunosuppression by UVR is cis-urocanic acid (UCA), which upon UVB irradiation is converted from the trans-form located within the stratum corneum of the epidermis (32). In mice, cis-UCA similarly to UVR suppresses the induction of CHS (24). Both UVR- and cis-UCA-induced suppression of CHS was reduced in mast cell deficient mice and in mice with capsaicin-impaired neurosensory system. However, cis-UCA is not capable of inducing mast cell degranulation by itself but induces the release of SP and CGRP from cutaneous sensory nerves (24), probably via stimulation of 5-HT2A receptors (33). This may lead to mast cell degranulation and the eventual release of mediators such as TNF-alpha, IL-10 and histamine. Histamine may then stimulate the keratinocyte production of prostanoids, which are important for UV-induced systemic immunosuppression (34).

Thus, it appears that UV-induced immunosuppression is closely related to the cutaneous neurosensory system and a mutual influence of mediators from nerves, keratinocytes, the stratum corneum (e.g., cis-UCA) and mast cells play significant roles in this process. How this is finally translates into antipruritic

effects of UVR is not yet known, but the aforementioned mediators involved in UV-induced immunosuppression, play also significant roles in neurogenic inflammation as well as in pruritus.

INTERACTION BETWEEN MAST CELLS AND SENSORY NERVES

In the skin, mast cells are located in close proximity to SP and CGRP positive sensory nerves (35). Mast cells are capable of releasing a number of preformed mediators such as histamine and tryptase as well as newly synthesized mediators such as neuropeptides (e.g., SP, CGRP, ET-1, VIP), cytokines (e.g., TNF-a, IL-4, IL-13, and IL-31) and lipid mediators (e.g., leukotriens and prostaglandins). This array of mediators interacts with their respective receptors on neighboring skin cells and sensory nerves, which upon stimulation may release neuropeptides such as SP and CGRP, which act back on mast cells as well as on other cells in the skin. Primary stimulation of sensory nerves and the eventual release of neuropeptides, on the other hand, stimulate the release of mediators from mast cells and other cells in the skin, which again affect cutaneouos sensory nerves. Thus, there is an intensive crosstalk between sensory nerves, mast cells as well as other cells in the skin via the aforementioned and other mediators and their receptors [for review see (35)] and they may participate in the antipruritic effects of UVR (**Figure 1**).

In lesional skin of AD (36) as well as psoriasis (37) the number of sensory nerve fibers positive for SP and CGRP as well as the number of cutaneous mast cells is increased. In addition, also the contacts between mast cells and SP/CGRP-positive nerves are increased, indicating an intensified crosstalk between nerves and mast cells in AD and psoriasis. Both have a high prevalence of chronic pruritus, especially in lesional skin, and respond well to phototherapy. In the skin of psoriatic patients suffering from pruritus an overexpression of the neuropeptide receptors for SP (NK1R) and CGRP (38) as well as of NGF and its high affinity receptor Trk-A (39) was found. A topical inhibitor of Trk-A, CT327, has shown significant antipruritic effects in psoriatic patients, indicating the importance of NGF for pruritus in psoriasis (40). Similarly, in AD patients an increase in NGF expression and cutaneous nerve fiber density was found. PUVA treatment resulted in downregulation of NGF and decrease of nerve fiber density, as well as in reduction of itch and eczema in these patients (18).

In uremic pruritus patients a papillary dermal "neuropathy" resulting from reduced CGRP+ papillary nerves was observed, which correlated negatively with pruritus intensity, suggesting a preferential loss of pain-sensing CGRP+ papillary nerves. SP+ and natriuretic polypeptide precursor B positive (NNPB+) nerve fibers, however, were preserved and the authors suggested SP+ and NNPB+(CGRP negative)–nerve fibers to be important itch-sensing candidates (41). There was no reduction in intraepidermal nerve fibers in ESRD patients with or without pruritus compared to non-ESRD controls arguing against a small fiber neuropathy causing pruritus in these patients (42).

Wallengren and Sundler reported that in 10 patients undergoing UVB/A, PUVA, or NB-UVB, for different skin diseases a decrease in intra-epidermal PGP9.5–positive nerves and dermal CGRP-positive nerves was shown, but nerve fibers for the vanilloid-receptor 1 (VR1) were not affected (43). They postulated that the reduction in nerve fibers by phototherapy may be responsible for the reduction of itch detected in these patients.

This is in discrepancy to the aforementioned increase in SP/CGRP-positive cutaneous nerve fibers by repeated suberythemogenic UVB irradiation in mice (27, 28) as well as to the hypothesis of Du et al. (41), that a reduction of CGRP+ nerves in the papillary dermis may participate in uremic pruritus. An increase in intraepidermal nerve fibers, SP and CGRP, as well as NGF, but a reduction of NK1R was also found in chronically sun-exposed skin by Toyoda et al. (44). Thus, there are conflicting results about a decrease or an increase in the number of cutaneous nerve fibers after repeated (suberythemogenic) UVR or phototherapy in mice and humans.

An increased number of mast cells was also found in the skin of patients with uremic pruritus. *In-vitro* experiments, showed an increased apoptosis of mast cells by BB-UVB and NB-UVB, suggesting a role of UV-induced MC-apoptosis in the antipruritic effect of phototherapy, at least in uremic pruritus (45). Indeed, a decrease in the number of mast cell as well as in pruritus after 2 months of UVB treatment was found in patients with uremic pruritus by Cohen et al. (46), however, the authors did not find a clear correlation between the reduction of mast cells and pruritus.

In urticaria pigmentosa, with a significant increase in mast cells in the skin of patients often accompanied with intense pruritus, PUVA is capable of reducing the number of cutaneous mast cells (47) as well as pruritus. In a study treating urticaria pigmentosa patients with high- and medium-dose of UVA-1, mast cells as well as pruritus also significantly decreased (48).

Taken together, it is not yet clear whether the change in the number of cutaneous nerves and/or mast cells is directly related to an antipruritic effect of phototherapy. It, however, shows, that UVR as applied by phototherapy is capable of affecting these two important players and thus affects pruritus, e.g., by mediators derived from them.

Endothelin-1 (ET-1) is such a mediator and neuropeptide. It is released from sensory nerves and by a number of skin cells including vascular endothelial cells, keratinocytes and mast cells, and is capable of inducing itch (49). In addition, stimulation of mast cells by ET-1, similar to SP, induces the release of several mediators such as histamine, leukotriens, IL-6, and TNF-a. On the other hand, ET-1 also stimulates the release of mast cell chymase, which degrades ET-1 and thus protects against ET-1 abundance, a condition which in mast cell deficient mice resulted in hypothermia, diarrhea and an increased death rate after systemic application of ET-1 (50).

Via this pathway, mast cells may even play an antagonistic effect against itch induced by UVR. Schweintzger et al. (51) have shown that, compared to normal mice, mast cells deficient KitW-Sh/W-Sh mice developed a specific photo-induced pruritus shortly after UV irradiation with doses well below inflammatory "sunburn" doses. Reconstitution of these mice with mast cells abolished this phenomenon of "photo-itch." The authors

explained this mast cell dependent UV-induced pruritus with an accumulation of ET-1 in the skin, induced by UVR (52), that resulted from an insufficient inactivation of ET-1 by the absence of mast cells-derived ET-1-degrading enzymes. The unopposed increase of ET-1 eventually may have stimulated cutaneous sensory nerves via their specific ETA receptors (49) causing the described photo-itch.

Other mast cells derived mediators may also stimulate pruritus. Beside mediators such as histamine, TNF-a, and IL-10, the enzyme tryptase is released upon mast cell stimulation and is capable of activating specific "protease activated receptors" (PAR2) on sensory nerve fibers or keratinocytes. By cleaving a tethered ligand of PAR, auto-activation of the receptor eventually causes the release of neuropeptides such as SP and CGRP, inducing neurogenic inflammation as well as pruritus (53). In AD, as aforementioned, the number of mast cells, SP- and CGRP-positive sensory nerves as well as NGF is increased (18, 36), and tryptase is upregulated. The release of tryptase from mast cells by NGF, eventually activating PAR2 on sensory nerves, thus, may also play a role in pruritus of AD (35).

ROLE OF CYTOKINES IN THE ANTIPRURITIC EFFECT OF PHOTOTHERAPY

Cytokines released from various cutaneous cells such as keratinocytes, Langerhans cells, mast cells, eosinophils and infiltrating lymphocytes are also suggested to be important mediators in chronic pruritus. Among these cytokines some are of specific interest.

In psoriasis, e.g., TNF-a, IL-17, and IL-23, are increased in the skin and may play a role in chronic pruritus of psoriatic patients. More than 80% of all patients suffer from chronic pruritus, and pruritus is the most distressing symptom of this disease (54). In clinical trials investigating anti-psoriatic treatments such as "biologicals" targeting these cytokines or their receptors (e.g., TNF-alpha or its receptor, IL-12/23p40, IL-23p19, and IL-17 or its receptors), beside an anti-psoriatic effect also a significant antipruritic effect of these drugs was detected. In addition, the "small molecules" such as phosphodiesterase 4 (PDE4) or Janus kinase (JAK) inhibitors have shown significant antipsoriatic as well as antipruritic effects. The reduction of pruritus by these biologicals or small molecules often paralleled or even preceded the reduction of psoriatic skin lesions (55).

Though the exact pathophysiology of pruritus in psoriasis is not yet known, it can be assumed that TNF-a, IL-17, and IL-23, may be involved. Indeed, e.g., the main receptor for IL-17A is found on many neural tissues and IL-17A participate in several neuroimmune interactions and directly or indirectly interact with neuronal functioning on the level of the DRG and the spinal cord. In addition, TNF-alpha may enhance the excitability of DRG neurons to other stimuli (56). In means of phototherapy, NB-UVB, the most frequently used phototherapy for psoriasis, has shown a significant downregulation of IL-17 in lesional as well as perilesional skin of vitiligo patients (57). In addition, PUVA therapy in psoriasis patients resulted in a significant downregulation of IL23 (IL12/23p40 and IL23p19). This indicates that phototherapy is capable of downregulating IL-17 as well as IL-23, and similarly to blockade of IL-17 or IL-23 with biologicals, this may contribute to the antipruritic effects of phototherapy, at least in psoriasis.

Another interesting cytokine is IL-31, which is primarily secreted by T-cells, mast cells, eosinophils, dendritic cells, and macrophages. Mast cell as well as eosinophil degranulation, e.g., by SP, may increase on-site IL-31 concentrations. IL-31, then binding to its receptor on sensory nerves can induce itch, and may also promote growth of nerves. It has been shown, that IL-31 induced pruritus is mediated via Transient Receptor Potential (TRP) receptors TRPV-1 and TRPA-1 (58).

In recent clinical trials, the IL-31Ra antagonist nemolizumab was capable of significantly reducing pruritus in AD (59) and in addition, improved atopic eczema. However, it is believed that IL-31 is also involved in pruritic conditions of other origin such as chronic prurigo, psoriasis, and cutaneous T-cell lymphoma (60). All of these conditions significantly respond to phototherapy and, thus, the question arises whether phototherapy also affects IL-31 or IL-31Ra. While acute high dose UVB is capable of transiently increasing IL-31 expression in the skin (61), UVA-1 phototherapy with suberythemogenic therapeutic doses for 6 weeks reduced IL-31 mRNA expression to levels close to normal, beside reducing atopic eczema and pruritus (62). In psoriasis, it has been shown that 20 repeated suberythemogenic NB-UVB treatments significantly reduced IL-31 serum levels (63). Thus, while acute high dose UVB increased IL-31 and pruritus, repeated lower doses of UVA-1 and NB-UVB appear to reduce IL-31 and pruritus, and it may be speculated that IL-31 reduction in the skin may contribute to the antipruritic effect of phototherapy in AD, in psoriasis, and maybe other pruritic conditions, e.g., chronic prurigo and CTCL, in which increased IL-31 or its receptor appear to play a role in chronic pruritus.

Other important interleukins, especially in AD, are IL-4 and IL-13, and it has been shown, that beside the aforementioned expression of IL-31, also IL-13 expression was reduced by UVA-1 phototherapy in AD patients (62). As aforementioned, the importance of IL-4 and IL-13 in AD was highlighted by the newly developed and already licensed antibody dupilumab, which targets the IL-4-receptor alpha-chain of the heterodimeric IL-4 and IL-13 receptors, and, thus, blocks both IL-4 and IL-13 mediated effects, which has shown significant antipruritic and anti-eczematous effects in AD patients (64). While both, IL-4 and IL-13, has been shown to directly stimulate a subset of DRG neurons *in vitro*, intra-cutaneous injection of IL-4 or IL-13 did not induce acute pruritic responses in mice (7). However, IL-4 enhanced neural responsiveness to multiple pruritogens such as histamine, chloroquine, thymic stromal lymphopoetin (TSLP) or IL-31. This increase in responsiveness to pruritogens was mediated via neuronal Janus kinase (JAK)-1. The authors reported that inhibition of JAK-1 by ruxolitinib or deletion of neuronal JAK-signaling in mice significantly reduced scratching in a murine AD model even in the presence of skin inflammation. In humans, tofacitinib, a JAK-1/3 inhibitor, significantly reduced pruritus in chronic idiopathic pruritus patients (7), who also favorably respond to phototherapy. The authors concluded that

IL-4, via neuronal JAK-1, is an important mediator of chronic pruritus as it "sensitizes" pruriceptive sensory nerves and lowers the threshold for other prurigenic mediators to induce itch. Interestingly, these authors also showed that like the activation of sensory nerves by IL-31, the TH2 cytokines IL-4 and IL-13 directly activate pruritic sensory nerves via TRP-channel dependent calcium influx.

Thus, the TRPV1 receptor, which is the classical capsaicin-receptors, appears to play a central role in mediating the effects of the important cytokines IL-31, IL-4, and Il-13, which seems to be crucial in chronic pruritus and eczema formation in AD, one of the major diseases treated successfully with phototherapy. In fact, it has been shown, that inhibition of TRPV1 receptors is capable of blocking pro-inflammatory effects of acute high dose UVR such as the induction of mRNA expression of the pro-inflammatory cytokines IL-1ß, IL-2, IL-4, and TNF-a as well as COX-2, indicating that UVR is indeed capable of affecting TRPV1 receptors (65). However, the effect of repeated suberythemogenic UVR, as used in phototherapy, on TRPV1 receptors is not yet known.

CONCLUSION

In conclusion, phototherapy has been shown to have significant antipruritic effects in various pruritic skin diseases in clinical trials and daily practice. Phototherapy also reduces pruritus in systemic diseases without primary skin lesions. Critical for the local or systemic antipruritic effect of phototherapy is the total area of skin irradiated, the number of UV treatments as well as the UV-dose. While high doses of UV result in sunburn and induction or aggravation of pruritus, repeated suberythemogenic UV doses are capable of inducing an antipruritic effect.

Despite the fact, that in recent years more and more information on possible mediators and receptors of chronic pruritus in various skin and systemic diseases became available, the exact pathophysiology of chronic pruritus in these diseases is not completely known, and at the moment our understanding about the possible mechanisms by which phototherapy conveys its antipruritic effect is very fragmented. Future laboratory and clinical investigations addressing the specific question, how repeated UV irradiation may affect cellular and neuronal structures and mediators involved in chronic pruritus, are necessary to combine the pieces of the puzzle to a clearer "image" of the antipruritic effect of phototherapy.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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REVIEW
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Extracorporeal Photopheresis—An Overview

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Extracorporeal photopheresis (ECP) has been in clinical use for over three decades after receiving FDA approval for the palliative treatment of the Sézary Syndrome variant of cutaneous T-cell lymphoma (CTCL) in 1988. After the first positive experiences with CTCL, additional indications have been successfully explored including areas such as graft-vs.-host disease (GVHD), scleroderma, and solid organ transplantation. The mechanism of action is still not fully resolved, but important steps in understanding ECP in recent years have been very informative. Originally, the primary hypothesis stated that psoralen and ultraviolet A (UVA) in combination induce apoptosis in the treated immune cells. This view shifted in favor of dendritic cell initiation, modification of the cytokine profile and stimulation of several T-cell lineages, in particular regulatory T-cells. A number of ECP guidelines have been produced to optimize treatment regimens in the clinical context. In CTCL, enough evidence is available for the use of ECP as a first line treatment for Sézary Syndrome (SS), but also as a second line or rescue treatment in therapy-refractory forms of mycosis fungoides (MF). ECP in the treatment of acute and chronic GVHD has shown promising results as second line therapy in steroid-refractory presentations. In solid organ transplantation, ECP has been used to increase tissue tolerance and decrease infections with opportunistic pathogens, attributed to the use of high doses of immunosuppressive medication. Infection with cytomegalovirus (CMV) remains a limiting factor affecting survival in solid organ transplantation and the role of ECP will be discussed in this review. A trend toward prophylactic use of ECP can be observed and may further contribute to improve the outcome in many patients. To further deepen our knowledge of ECP and thus facilitate its use in patients that potentially benefit most from it, future prospective randomized trials are urgently needed in this rapidly growing field. The aim of this review is to (1) introduce the method, (2) give an overview where ECP has shown promising effects and has become an essential part of treatment protocols, and (3) to give recommendations on how to proceed in numerous indications.

Keywords: ECP, ultraviolet A, CTCL, GVHD, scleroderma, solid organ transplantation

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INTRODUCTION

Extracorporeal photopheresis (ECP), also known as extracorporeal photoimmunotherapy or photochemotherapy, is a leukapheresis-based therapy which was initially used in patients with cutaneous T-cell lymphoma (CTCL) (1). Specifically for the treatment of therapy refractory CTCL patients suffering from the leukemic variant, the Sézary Syndrome, ECP received FDA (United States Food and Drug Administration) approval in 1988. During ECP, whole blood of

the patient is collected via a cubital vein, or a permanently implanted catheter, for separation of leucocytes from plasma and non-nucleated cells. With a specifically constructed device for this procedure, collected leukocytes, the so called buffy coat, are then exposed to ultraviolet-A (UVA) irradiation in the presence of a photosensitizing agent, 8-methoxypsoralen prior to reinfusion to the patient (**Figure 1**). Two basically different methods for performing ECP procedure have been described. They differ in the device used for leukocyte collection and UVA irradiation: the "closed system" and the so called "open system." The closed system is based on the original design by Edelson and coworkers and is the only FDA-approved system. The open system is a system incorporating different separation instruments, mostly used outside the United States. No prospective comparative studies have been performed. Although ECP is a valid treatment method since 30 years and over 2 million of treatments have been performed, there are no reports about negative cytogenetic effects. Petersheim et al. investigated the mitotic index (MI), type and number of chromosomal aberrations after ECP treatment and could demonstrate that ECP is not associated with an increased mutagenic risk (2).

Over the last decades, indications for initiating ECP were continuously extended since its introduction. ECP treatments are generally well-tolerated by patients and there are almost no significant unwanted side effects. Taken together, ECP combines an excellent safety profile with efficacy. The aim of this article is to (1) introduce this technology, (2) give an overview where ECP has been showing promising effects and has become an essential part of treatment methods, (3) and to give recommendations on how to proceed in multiple indications.

MODE OF ACTION

It has been 35 years since the first study on ECP was completed and 30 years since ECP was approved by the United States Food and Drug Administration (FDA). Nonetheless, the mode of action is still vaguely known, although many achievements have been made over the last decades. Research has shifted from mainly exploring new indications for ECP to a better understanding of the mechanisms of action in order to extend again the use of ECP for a wider range of diseases, but now with a clearer focus in mind (3).

Early studies ascribed the therapeutic effect of ECP to the initiation of apoptosis in lymphoid cells (4, 5). For this purpose, the photosensitizer 8-MOP was combined with exposure to UVA (320–400 nm), a concept which originally derived from the use of oral psoralen plus UVA (PUVA)-therapy but with the important difference that instead of 8-MOP-photosensitized skin (conventional oral PUVA therapy), buffy coat incubated with 8-MOP was exposed to UVA (ECP). UVA irradiation of cells after incubation with 8-MOP leads to DNA crosslinking. After reinfusion, subsequent apoptosis of lymphoid cells, largely natural killer (NK) cells and T-cells, arises (6).

While these proposed mechanisms might explain the therapeutic effect of ECP on CTCL, it does not elucidate how

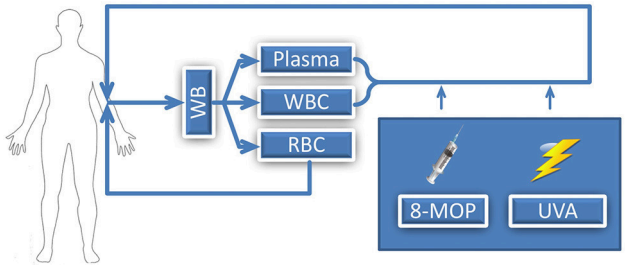

FIGURE 1 | Illustration of ECP procedure; WB, whole blood; WBC, white blood cells; RBC, red blood cells; 8-MOP, 8-methoxypsoralen; UVA, Ultraviolet A.

ECP should work in other indications. Hence, researchers' view on possible mechanisms of action shifted to a merely immunomodulatory approach. In line, a recently published consensus of the American Council of ECP underlines the importance of dendritic antigen-presenting cells (DCs) in the mechanisms of action of ECP (3).

Activation of monocytes occurs after contact with extracorporeal surfaces, which can be found in the tubing and the radiation chamber of the ECP device. Activated monocytes differentiate to immature DCs (iDCs) and consecutively get loaded with patient-specific antigens. These cells show characteristic surface markers of iDCs (CD83, X-11, Alpha-V, Beta-V, CD1a) (3, 7–10). The mechanism promoting differentiation to iDCs seems to relate to direct UVA effects and/or exposure of the buffy coat to extracorporeal surfaces (11). Upon reinfusion, phagocytosis of lymphoid cells is performed by iDCs, which subsequently undergo maturation and present antigenic peptides. This process has been named transimmunization (12).

It has been observed that the cytokine composition in the peripheral blood (increase of TNF-alpha and IL-6) changes after reinfusion of 8-MOP and UVA treated cells into the patient (13). An increase of CD36+ macrophages, due to the changes in tumor necrosis factor (TNF)-alpha and interleukin (IL)-6 levels, can be found after ECP. Hence, an immune response shift occurs which normalizes the imbalance of the Th1/Th2 response that can be found in CTCL. Summarizing, anti-inflammatory cytokines may be induced by ECP, whereas pro-inflammatory cytokines may be reduced (14, 15). As this may be beneficial for CTCL, the effect in autoimmune diseases must follow a different pathway. Indeed, in patients with graft-vs.-host disease (GVHD), ECP shifts the cytokine profile toward a Th2 immune response. Comparing the cytokine profiles before and after ECP in these patients, an increase of IL-4, IL-10 and transforming growth factor (TGF)-beta and a decrease of IL-12, IL-1, interferon-alpha, and TNF-alpha was observed, resulting in the apoptosis of mononuclear cells (16, 17).

Activation of T-cells leads to a differentiation into several cell lineages, particularly regulatory T-cells (Tregs) playing an important role in the down-regulation of immune reactions. Especially in patients with acute GVHD (aGVHD), Treg differentiation after ECP is highly reinforced and a significantly

higher number of Tregs is noticeable in the peripheral blood in GVHD patients after ECP (18, 19). In a murine model 8-MOP and UVA-treatment induced Tregs similar to UVB-induced antigen specific Tregs characterized by the expression of CD4, CD25, CTLA-4, and Foxp3. In addition, it has been demonstrated that IL-10 is involved in this process (20–22). ECP might highly efficiently stimulate Tregs as has been shown in a murine model by Gatza et al. (18), Rezvani et al. (23), Zhai et al. (24), and Wolf (25). In the area of solid organ transplantation, ECP has been gaining more and more acceptance. In lung transplanted patients, a slight up regulation of CD4+CD25+Foxp3+ Tregs has been reported, possibly contributing to an increased immunotolerance of transplanted tissues and organs and hence survival rates (26).

In summary, research shifted from apoptosis induced by exposure to psoralen with UVA to an immunomodulatory approach, which is based on the initiation of dendritic cells, a modification of the cytokine profile and the stimulation of several T-cell lineages, in particular regulatory T-cells. Nonetheless, different pathways contribute to the beneficial effects of ECP in different indications and the final role of regulatory T cells has yet to be definitively established.

INDICATIONS

Cutaneous T-Cell Lymphoma (CTCL)

Cutaneous T-cell lymphoma (CTCL) represents a lymphoproliferative disorder primarily characterized by skin involvement due to accumulation of malignant T-cells. The most common subtypes of CTCL are mycosis fungoides (MF) and Sézary Syndrome (SS), which account for more than half of all CTCL patients. MF often resembles eczema or psoriasis in an initial phase, but is characterized by a clonal T-cell population. Patients often suffer from itchy plaques, but with disease progression nodular lesions and tumors may appear. In SS atypical mononuclear cells with a cerebriform nucleus (Sézary cells) appear which can be found in the skin, peripheral blood and lymph nodes. SS usually has a bad prognosis with a 5-year survival rate of 24% (27, 28). Initial treatment of CTCL is directed at the cutaneous involvement to improve quality of life and minimize the risk of reoccurrence. With disease progression, the addition of immune modulatory treatments, chemotherapy or stem cell transplantation may become a necessity (28, 29).

The first investigational study using ECP was performed in patients with the leukemic variant (Sézary Syndrome) of CTCL. In a meta-analysis for the efficacy of ECP, a response rate of 55.7% and a complete remission rate of 17.6% could be reported (1). A better response rate was noticed in patients with a low count of Sézary cells and low CD4/CD8 ratio. Patients with a low number of CD4+CD7-cells may also have a higher benefit from ECP. A combination of ECP with immune modulatory treatment may enhance the benefit of ECP (28, 30, 31). With the leukemic variant of CTCL as the oldest indication for ECP, many studies support the first-line use of ECP. A combination therapy can also be performed, with optimal response being attributed to the combination of ECP, interferon-alpha and bexarotene (31).

ECP has been established as a first-line treatment in CTCL patients with blood involvement (stage IVA1 or IVA2) and erythrodermic stage IIIA or IIIB (30, 32, 33). Treatment recommendations stated 2-weekly cycles of treatment on 2 consecutive days for at least 3 months and subsequent treatment every 3–4 weeks. Re-evaluation of treatment response should be performed between months 6 and twelve. If response is seen, treatment should be continued every 4–8 weeks. Combination of therapies can be considered, if ECP fails as first-line treatment (31, 34).

Graft-Vs.-Host Disease (GVHD)

Although allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative treatment of hematologic diseases, GVHD is still a limiting factor for the outcome of these patients (35). With possible involvement of multiple organs such as the skin representing the most common appearance, GVHD in liver, gut and in rare cases in lung and neuromuscular system are reported. According to the Consensus of National Institute of Health further sub-classification can be done into acute and chronic GVHD (36, 37). Corticosteroids remain first-line therapy for both acute and chronic GVHD but due to its association with significant toxicity and an increasing number of patients developing steroid-refractory disease, many salvage therapies are currently available. Based on recently published literature, mammalian target of rapamycin (mTor)-inhibitors (Sirolimus), janus kinase (JAK)-inhibitors (Ruxolitinib), proteasome inhibitors (Bortezomib), and also interleukin (IL)-22 are showing promising efficacy in the treatment of GVHD (38). For the treatment of chronic GVHD, Ibrutinib, an irreversible inhibitor of Bruton's tyrosine kinase (BTK), and Interleukin-2 inducible T-cell kinase (ITK), was recently granted FDA approval and is currently the only one approved for this purpose (39).

ECP is a widely recommended treatment modality as a second-line treatment, particularly in steroid-refractory form of GVHD. Current recommendations indicate that treatment should be performed on 2 consecutive days every week or every 2 weeks until a response is noticeable. ECP Treatments should be continued for at least 8 cycles or until complete remission is occurring (40). In a retrospective multicenter analysis, ECP has shown response rates of 80% in acute and chronic GVHD patients (41). A meta-analysis reviewed 7 prospective studies on acute GVHD and found overall good response rates but also a necessity of further prospective controlled multicenter studies (42). In a recently published article, the use of ECP as an initial prophylactic treatment was discussed, indicating its beneficial effect (43). An uncontrolled, prospective trial was able to show promising results for prophylactic use which has still to be confirmed in future studies (44).

Scleroderma

Scleroderma is an autoimmune connective tissue disease characterized by increased fibroblast activation leading to hypertrophic dermal collagen. Skin involvement is just one appearance, beside joints and internal organs. Scleroderma is usually subdivided into a systemic (generalized) and a more localized form Zhou and Choi (45) and Gabrielli et al. (46). The pathogenesis of scleroderma is not well understood, however,

Th2 and Th17 cells with accompanied cytokines, together with changes in number and function of Tregs might be related to the development of scleroderma (45, 47–49). Current treatment is based on immunosuppression, which include topical and systemic steroids, azathioprine, cyclophosphamide, methotrexate, mycophenolate mofetil (MMF), or interferons. Phototherapy is also a major component in the treatment of scleroderma and ranges from narrowband to broadband UVB, UVA, UVA1, PUVA, and ECP (50).

The use of ECP for scleroderma has been investigated in single patients with refractory disease (51, 52). A few larger treatment series are available. Treatment regime was usually performed on 2 consecutive days with a re-treatment every 2–6 weeks with a follow-up of usually 12 months. The effect of ECP was also investigated in randomized, double blind, placebo controlled studies with varying outcome, ranging from no improvement against no treatment, improvement over no treatment but no improvement against sham to a superiority of ECP against D-penicillamine treatment (53–60). Patients with scleroderma may have a higher risk in developing lung cancer, but no difference was found between patients with ECP and patients without ECP treatment (61).

Concluding the results of the published studies, best evidence of the use of ECP in scleroderma is given for skin manifestations, although joint involvement may also benefit. Scleroderma is an indication for ECP with a category III (grade 2B) by the American Society of Apheresis. This is supported by other guidelines which identify ECP as a second-line or alternative treatment in refractory patients (34, 62).

Solid Organ Transplantation

Based on recently published statistical data from Eurotransplant, ∼5,500 transplantations of solid organs were performed in 2017, with an ever continuously increasing number (63). Although major improvements in surgical techniques and new immunosuppressive protocols have been made, the long-time survival of transplanted patients is still limited due to acute and chronic allograft rejection, as well as opportunistic infections.

The first investigational study using ECP in the field of solid organ transplantation was performed in cardiac transplant rejection in 1992. By assessing endomyocardial biopsies after ECP treatments, successful reversal of acute cardial rejection could be observed (64, 65). Further studies in heart transplant recipients suffering from acute or chronic rejection were able to prove efficiency of ECP in reducing frequency and degree of rejection severity, without higher incidence of infections (66–69). In one study a significant reduction of cardiac allograft vasculopathy (CAV) in the ECP group determined by intravascular ultrasound was demonstrated (70).

Similar results by initiating ECP in the lung transplantation setting could be documented. Several trials presented efficient clinical response in the treatment of chronic rejection. Benden et al. examined the use of ECP in patients with bronchiolitis obliterans syndrome (BOS) and recurrent acute rejection after lung transplantation and were able to demonstrate that ECP reduced the rate of decline in lung function in BOS patients. In addition, patients suffering from recurrent acute rejection were clinically stabilized (71). Jaksch et al. were able to confirm the clinical improvements in BOS patients showing stabilization of lung function and significant greater survival (72). Greer et al. performed a retrospective analysis of all patients treated with ECP for chronic allograft dysfunction demonstrating stabilization as well as improvement in forced expiratory volume in 1 s (FEV1) (73). A recently published meta-analysis emphasizes the beneficial effect of ECP for clinical improvement of BOS (74). Nonetheless prospective, randomized controlled studies with a larger cohort are still missing to validate these results.

Several trials have been performed using ECP in the treatment of acute and chronic rejection after solid organ transplantation, though there is only one study examining the effect of ECP in prophylactic use. Cardiac transplant recipients were randomized to receive standard triple immunosuppressive therapy or additionally ECP treatments within the first month of transplantation. Promising results could be detected in the prevention of chronic rejection by decreased levels of non-donorspecific panel reactive antibodies (PRA) and decreased coronary artery intimal thickness in the ECP treated group (70). Data on using ECP as prophylaxis for allograft rejection in lung transplantation recipients is still missing and currently a highly relevant topic.

Recommendations are well established for patients suffering of BOS after lung transplantation and ECP treatment should start as soon BOS is diagnosed. In heart transplantation, ECP can be considered as an additional treatment. Cycles should be performed on 2 consecutive days with one cycle every 2 weeks for 3 months. After this initial phase, treatment intervals can be prolonged to once every month. It is still unclear how long ECP treatment should be continued, with ranges of 6–24 cycles. Continued treatment may be helpful in good responding patients with an improvement of clinical function (i.e., FEV1 in lung transplantation) (34).

Crohn's Disease

Crohn's disease (CD) represents an inflammatory condition, which can affect the entire gastrointestinal tract. This topographic distinction is often used to separate CD from ulcerative colitis, which mainly affects the colon, although the terminal ileum and colon are also primary affected by CD. Complications of the disease range from stricturing to penetrating complications after chronic inflammation. Intestinal surgery is often initiated after serious complications (75). The disease arises from hyperimmunity and chronic inflammation of the mucosa (76). It is therefore reasonable, that immunosuppression, such as steroids, methotrexate, TNF-alpha blockers, and other agents are a major component in the treatment of the disease. When using monotherapy or combined immunosuppression, the risk of infections are usually a limitation and restrict treatment success (77).

The use of ECP in CD is still not well established. In a pilot study with treatment on 2 consecutive days every 2 weeks for 12 cycles, a withdrawal from steroid therapy in almost half of the ECP treated patients could be reached, without relapsing symptoms. In almost all other patients, steroid dose could be

reduced by at least half of the initial dose (78). In uncontrolled prospective studies, ECP was well tolerated and clinical response was initiated in half of the patients with a remission rate up to 25% and a significant reduction of steroid doses (79, 80). The use in pediatric patients is an unexplored area, but a case report is in accordance to the results seen in adults (81).

Atopic Dermatitis

Atopic dermatitis (AD), also known as atopic eczema, is a chronic relapsing skin disease, mainly characterized by itchy skin lesions. Severity is often represented by the affected area of the skin (82–84). Skin lesions of AD are histologically characterized by epidermal changes. These include spongiosis and epidermal hyperplasia, combined with dermal infiltrates consisting of T-lymphocytes, monocytes, and eosinophilic cells. A genetic background is often involved in this multifactorial disease (85). On a cellular level, a malfunction of Tregs and an impaired Th2/Th17-driven immune response to antigens can be observed, that leads to skin changes (86, 87). Standard therapy for adults usually includes topical steroids, calcineurin inhibitors, or phototherapy (i.e., UVA-1, PUVA, or UVB). In refractory cases, systemic therapy becomes a necessity. Promising results have been achieved using the IL-4 receptor antagonist dupilumab, which has been approved by the EMU/FDA in 2017 (88, 89). In selected severe, otherwise refractory cases, the use of rituximab or intravenous IgG (IVIG) might be an option.

The use of ECP for AD has already been performed for almost 25 years with the first publication in 1994 by Prinz et al. (90). After these initial three patients with good response, several open label studies were conducted that proof usefulness of ECP in standard therapy refractory AD patients with a significant decrease of affected skin area (91–99). Although the clinical effect of ECP in AD is limited, patients with refractory disease might benefit from ECP in combination with topical or systemic treatment.

Type 1 Diabetes

Type 1 diabetes (T1D) is a T-cell mediated autoimmune disease where T-cells are directed against pancreatic insulin-producing beta-cells. Management of this disease is usually performed with blood glucose control self-monitoring and insulin injections. Severity can be graded on the remaining beta-cell function. The lower the remaining insulin production, the higher the risk of long-term complications (100, 101). Because beta-cell function is a vital predictor of disease severity, the preservation of these cells plays a crucial role in the management of this disease. Evidence shows that beta-cells have a regenerating ability (102). The exact autoimmune pathogenesis remains vague, but it is evident, that autoreactive CD4+ and CD8+ T-cells play an important role in the destruction of pancreatic beta-cells, whereas other autoantibodies may also be involved in this process (103). Summarizing the conditions in T1D, an imbalance of the immune system is occurring and the solitary suppression of the immune response does not seem adequate, considering the adverse events (104–106).

In a non-obese diabetic mouse model, cells treated with ECP were reinfused and the development of T1D was significantly delayed. An immune regulatory process is likely to occur in this scenario and Foxp3+ Tregs may be involved (107). Only one study is available, where ECP was used in newly diagnosed T1D patients. The group of children treated with ECP produced more C-peptide and needed significantly lower doses of insulin per kg bodyweight (82).

In conclusion, few studies are available for the evaluation of usage of ECP for T1D, but published data shows promising results as an additional therapy to delay the onset of T1D. Because ECP was well tolerated in the clinical trial, further studies on young patients may improve the outcome of this autoimmune disease.

CONCLUSION

Since the first prospective trial on the use of ECP was performed by Edelson et al., multiple promising results in various entities have been published in the last decades. ECP found its establishment in the treatment of different diseases and acceptance as an immunomodulatory therapy with high potential of inducing tolerance. To date, no significant side effects have been reported. Due to its excellent safety profile, ECP is more and more investigated in prospective randomized trials with larger cohorts—on the one hand to extend its clinical indication with a clearer focus, and on the other hand to examine the complexity of the underlying immunomodulatory mechanism of action. Further research on identifying biomarkers which could predict the response to ECP is required.

AUTHOR CONTRIBUTIONS

AC designed a concept, performed literature search, and wrote the manuscript. CJ gave additional ideas and performed correction of manuscript. RK performed supervision and final correction.

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PERSPECTIVE
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A Perspective on the Interplay of Ultraviolet-Radiation, Skin Microbiome and Skin Resident Memory TCRαβ+ Cells

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The human skin is known to be inhabited by diverse microbes, including bacteria, fungi, viruses, archaea, and mites. This microbiome exerts a protective role against infections by promoting immune development and inhibiting pathogenic microbes to colonize skin. One of the factors having an intense effect on the skin and its resident microbes is ultraviolet-radiation (UV-R). UV-R can promote or inhibit the growth of microbes on the skin and modulate the immune system which can be either favorable or harmful. Among potential UV-R targets, skin resident memory T cells (T_{RM}) stand as well positioned immune cells at the forefront within the skin. Both $CD4^+$ or $CD8^+$ αβ T_{RM} cells residing permanently in peripheral tissues have been shown to play prominent roles in providing accelerated and long-lived specific immunity, tissue homeostasis, wound repair. Nevertheless, their response upon UV-R exposure or signals from microbiome are poorly understood compared to resident TCRγδ cells. Skin T_{RM} survive for long periods of time and are exposed to innumerable antigens during lifetime. The interplay of T_{RM} with skin residing microbes may be crucial in pathophysiology of various diseases including psoriasis, atopic dermatitis and polymorphic light eruption. In this article, we share our perspective about how UV-R may directly shape the persistence, phenotype, specificity, and function of skin T_{RM}; and moreover, whether UV-R alters barrier function, leading to microbial-specific skin T_{RM}, disrupting the healthy balance between skin microbiome and skin immune cells, and resulting in chronic inflammation and diseased skin.

Keywords: skin microbiome, ultraviolet-radiation, skin resident memory T cells, inflammation, immune suppression, photomedicine, phototherapy

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INTRODUCTION

Skin Microbiome

Human skin with its large surface (1) harbors a wide variety of microbes, which include bacteria, fungi (2), viruses (3, 4), archaea (5, 6) and skin mites (4, 7, 8). These microbes exist in either a mutualistic and/or competitive relationship with each other (microbe-microbe) (9) and the host (10–13). Commensals make up for most of the microbiome followed by opportunistic and/or

pathogenic microbes. The diverse physical nature of the skin with its variable water content, pH, lipids and sebum quantity among others crucially influence the diversity of the microbiome. However, it is intriguing that myriads of microbes reside on the skin surface (**Figure 1**) as well as in sub-epidermal compartments (14), despite the robust nature of the skin's immune system to rapidly detect and neutralize any foreign intruders (15). Many common cutaneous conditions such as atopic dermatitis (AD), psoriasis and rosacea are associated with dysbiosis of skin microbiome, most commonly driven by commensal species. A recent review highlights the latest findings regarding the microbial interactions with the immune system and microbial composition in health and diseases such as AD, acne, chronic wound infections, and primary immunodeficiencies (16).

Ultraviolet-Radiation (UV-R)

UV-R is one of the most prominent external factor affecting the skin (17) and the microbiome (8, 18, 19). UV-R mediated immune suppression was first discovered by Kripke et al. (20). This was further confirmed and proved to be T-cell mediated by using contact hypersensitivity (CHS) models in mice (21) and in humans (22–24). The initial key events that are prominently involved in immune suppression after UV-irradiation are DNA damage (25), formation of reactive biophospholipids like platelet activating factor (26) and isomerization of inactive *trans*- to active *cis*-urocanic acid (UCA) (27). A study conducted by Kubica et al. (28) used caspase-14 deficient mice which are known to have reduced levels of UCA and observed significant alterations in the skin microbiome. It is intriguing that caspase-14 is involved in proteolysis of filaggrin which is the major source of UCA in the skin and mutations in filaggrin are linked to the development of AD which is in turn linked to an altered microbial landscape (29). Certain skin commensals such as *Micrococcus luteus* can degrade cis-UCA to its trans isoform (30) and thus potentially diminish immune suppression. An early report from our group suggests that cis-UCA can indeed

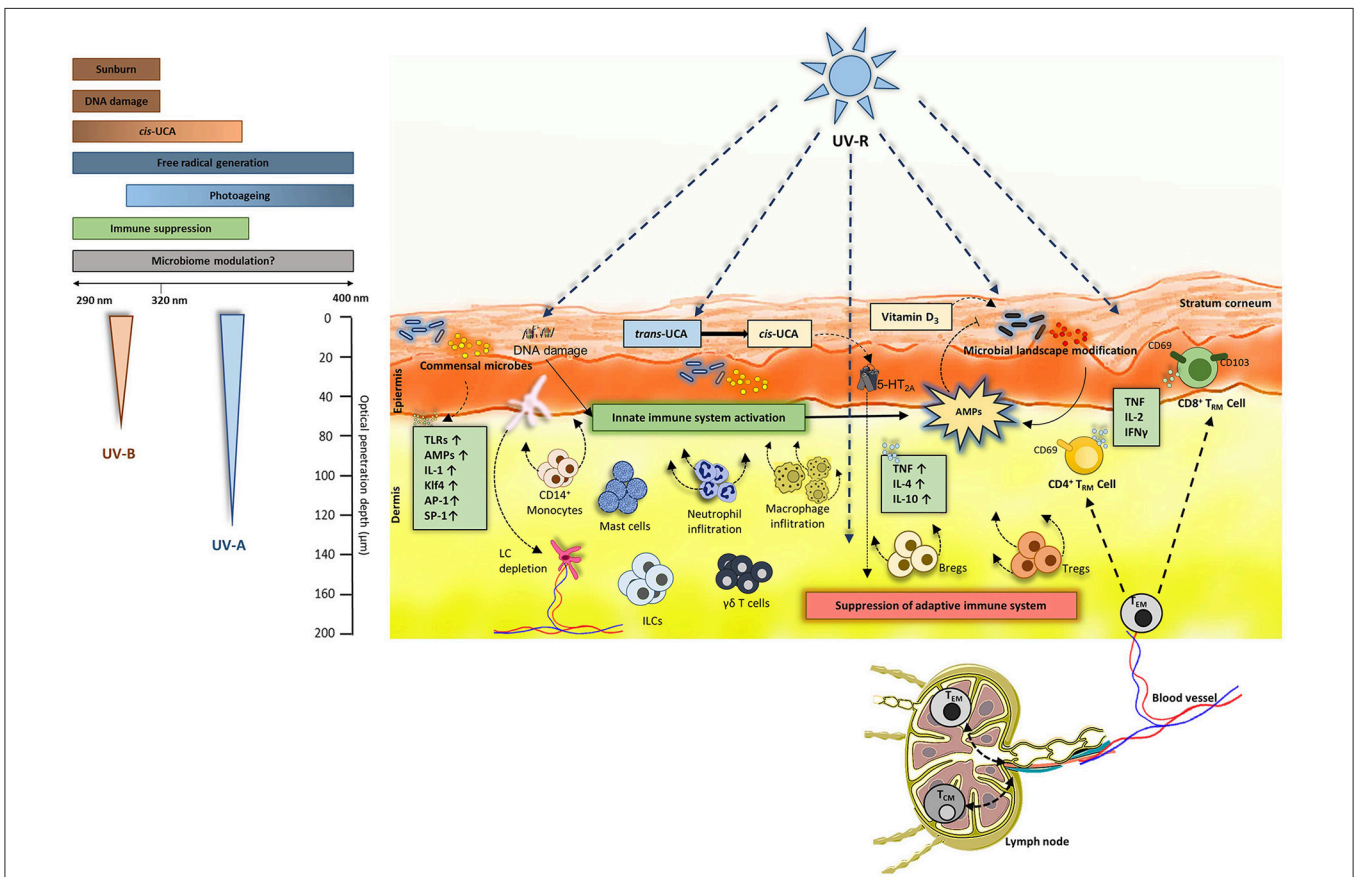

FIGURE 1 | UV-induced events in the skin: Both UV-B (290–320 nm) and UV-A (320–400 nm) penetrate the skin. UV-B causes sunburn and DNA damage and is also known to induce immune suppression. UV-B and UV-A (to some extent) converts trans-UCA to cis-UCA and generates free radicals. Commensal microbiome colonizes the skin and can induce production of various cytokines, antimicrobial peptides (AMPs) and activate toll-like receptors (TLRs). The effects of UV-B and UV-A on skin microbiome is not fully understood. Overall, UV-R is known to activate innate immunity by production of AMPs and by stimulating innate cells like macrophages, mast cells, innate lymphoid cells (ILCs) and skin resident γδ T cells. On the other hand, UV-R induces an immune suppressive environment in the skin by inducing production of TNF, IL-4, IL-10. As overall result, regulatory T cells (Tregs) and B cells (Bregs) are induced leading to functional immune suppression and subsequent inhibition of effector T cells present in the skin. Regarding TCRαβ^{+} lymphocytes, effector memory T cells (T_{EM}) can circulate between the blood, lymph and skin where they receive environmental signals. Also, the dermis is populated by CD4^{+} T_{RM} (CD69+ CD103±) whereas the epidermis is composed of CD8^{+} T_{RM} (CD69^{+}CD103$^{\pm}$) in majority. These T_{RM} populations can produce TNF-α, IL-2 and IFN-γ depending on the microenvironment. Nevertheless, the UV effect on these T_{RM} remains to uncovered.

directly modulate skin microbiome (31). Since UV-R suppresses the immune reaction to antigens of infectious microbes such as *M. lepraemurium*, *bovis BCG*, *C. albicans*, *B. burgdorferi*, and *Schistosoma mansoni* (32–34) it can be speculated that exposure to UV-R could enhance susceptibility to infections, however clinical evidence of increased infections after UV-R is very low. This could be due to the fact that UV-R suppresses adaptive immunity but activates innate immunity (35). One of the important innate key players are antimicrobial peptides (AMPs). These are small proteins typically ranging from 10 to 50 amino acid residues that have potential to neutralize invading microorganisms (36) and mediate adaptive immune response (37–39). Dysregulation in AMP expression could be linked to many diseases, including photosensitive conditions like polymorphic light eruption (PLE) (40), where AMPs may be key mediators to maintain homeostasis between host immune system and microbiome. UV-R exposure also leads to infiltration of macrophages and neutrophils (41–43), induces emigration of Langerhans cells (LC) from the skin into the draining lymph nodes (44–46) and affects mast cells. Furthermore, regulatory T cells (Tregs) and B cells (Bregs) are recruited and activated (47, 48). All these cells and UV-induced events are known to be involved in immune suppression (49) (**Figure 1**). It has been known for a long time that UV-induced immune suppression is mediated by T cells (21, 50), however, the exact role of UV effects on the more recently described T_{RM} and immune function are largely unexplored.

Skin-Resident Memory T Cells (T_{RM})

Among all the immune cells present in the skin, such as dendritic cells, macrophages, γδ T cells and NK cells, T_{RM} (51) are now considered as key players of immunity (52–54) (**Figure 1**). They have been described in various tissues such as skin, lung, gut, liver and brain (55–57). T_{RM}, along with effector and central memory T cells (58), are either $CD4^+$ or $CD8^+$ T cells that are derived from naïve specific T cells which were activated upon a previous immune response. Thus, T_{RM} share a common clonal origin with central memory T cells (59) but diverge in terms of dynamics, phenotype, and function. The major characteristics of T_{RM} are their capacity to survive and stay poised in the skin for a long time (60) as well as play a key role for pathogen clearance and immune alert (53). In other words, T_{RM} do not recirculate in the lymph or blood but rather patrol in the skin. $CD8^+$ T_{RM} are more localized in epidermis whereas $CD4^+$ T_{RM} populate preferentially the dermis (61). This non-recirculating pattern is conferred by the expression of CD69 which blocks sphingosine-1-phosphate receptor (S1P1), a receptor normally allowing lymph entrance. Moreover, a significant part of skin T_{RM} express CD103, the α-chain of the integrin αEβ7 which interacts with E-cadherin expressed by keratinocytes. Once arrived in the skin, killer-cell lectin like receptor G1 (KLRG1)-T_{RM} precursors receive key signals for their establishment in the tissue. Among them, TGF-β is a critical signal integrated by T_{RM} via TGF-βRII (52) and required for their residency. TGF-β can notably be produced by keratinocytes which thus play a role on T_{RM} retention (62). TGF-β alone is not sufficient for skin T_{RM} establishment, but rather acts in combination with other cytokines expressed in the skin such as TNF-α and interleukin (IL)-33 (63). Moreover, hair follicles seem to play a role on the recruitment and establishment of skin T_{RM} notably through the production of IL-15 and IL-7 (**Figure 2**) (64). Apart from cytokines, lipids available in the skin are key for T_{RM} maintenance (65). Functionally, T_{RM} allow a faster immune response upon pathogen entry through the production of alarmins such as IFN-γ and chemokines to recruit neutrophils, monocytes as well as circulating memory T cells on the site. T_{RM} are also able to proliferate locally after a recall response to maintain themselves (66). Finally, T_{RM} are able to be strongly cytotoxic (67).

UV-INDUCED IMPACT ON SKIN TRM

At least $1–2 \times 10^{10}$ resident T cells comprising T_{RM} populate the human skin (68, 69), and it is highly logical that they experience similar impacts from UV-R as the other immune cells. These sentinel cells have numerous essential functions within the skin for cutaneous immunity and repair along with wound healing, antimicrobial responses and local tissue inspection (68, 70–72). The impact of UV-R on immune response mediated by T cells such as $CD4^+$, $CD8^+$, and Tregs has been previously described (73–75), however, the effects of UV-R on shaping the persistence, phenotype and specificity of skin T_{RM} are poorly understood. It is therefore important to understand the interaction between the skin T_{RM} and UV-R in mediating UV-induced immune suppression. It is thought that after an acute UV-exposure, the damaged keratinocytes release ATP (76) and ATP-mediated IL-1 (77) in an accelerated way; furthermore, this extracellular ATP is thought to be involved in adaptive immune responses (78, 79). Moreover, UV-R upregulates CD69 expression on TCRγδ cells (77) and could exert a similar effect on skin T_{RM} for which CD69 is crucial for their residency in the tissue. Besides, in the absence of γδ T cells, there was reduced DNA repair of UV-induced lesions in mice, suggesting the role of these γδ T cells in the repair (77). Such a role for T_{RM} has been demonstrated in acute wounds (71) but needs to be addressed in the case of UV-induced damage. T_{RM} may have long been unknown targets of UV-phototherapy in diseases which are now understood as T_{RM} cell-mediated (80). Patients with cutaneous T cell lymphoma (mycosis fungoides) are known to have malignant T cells that lack L-selectin and CCR7 expression, a phenotype that is similar to T_{RM} (81). The common treatment modality for these patients include phototherapy (82) and low-dose radiation. However, the effects of phototherapy on T_{RM} is completely uncharacterized (83).

INFLUENCE OF SKIN MICROBIOME ON SKIN T_{RM}

The skin is exposed to a large number of microbes throughout the lifetime, of which only a minor proportion is pathogenic. It has been suggested that the primary purpose of the immune cell memory is to maintain the immune homeostasis with the commensal microbes (84). Recent studies in various mouse models and in humans show that the composition of the

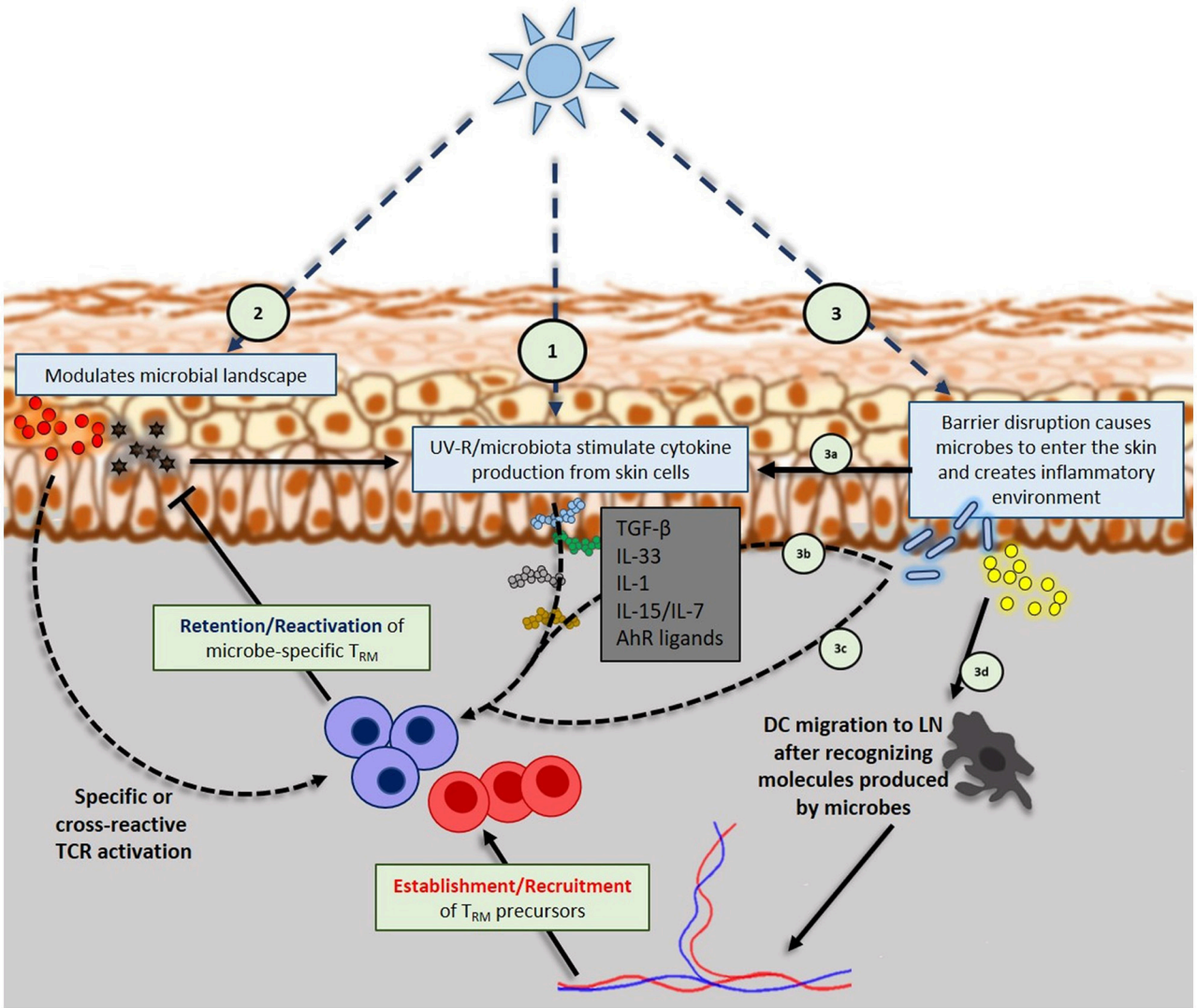

FIGURE 2 | Interplay of UV-R, skin microbiome and skin resident memory TCRαβ+ cells: (1) UV-R induces keratinocytes and other skin cells to produce inflammatory or regulatory cytokines that will influence T_{RM} phenotype, retention and reactivation. (2) UV-R modulates microbial landscape, eventually releasing microbial antigens into the skin that will be up taken by dendritic cells (DC) that will specifically activate T_{RM} (regulatory or effector). Microbial antigens can also trigger the production of inflammatory cytokines by keratinocytes that further activate T_{RM}. (3) High doses of UV-R can cause barrier disruption that will allow skin resident microbes to enter the skin; danger signals from barrier disruption (3a) and microbes entered into the skin (3b) will trigger cytokines production by keratinocytes, DCs, ILCs, NK and TCRγδ cells. Those cytokines will take part in shaping T_{RM} phenotype and activation. Entered microbes can also activate skin T_{RM} in a specific manner (3c) or be uptaken by DCs (3d) in order to activate naïve specific T cells in draining lymph nodes that will be recruited on the site.

skin microbiome is crucial in mediating appropriate immune responses toward a pathogen and in maintaining the normal immune status in the skin (10, 11, 15, 28, 85–87). Whether certain species of commensal microbiome influence the type of T_{RM} within the skin is not known, but a lot can be learnt from the gut. In one of the studies using mice, commensal specific memory T cells were found in the intestines (88) and similar T_{RM} cells could exist in the skin as well. Both memory $CD4^+$ and $CD8^+$ T cells can act against infections with influenza virus (55, 89), lymphocytic choriomeningitis virus (90, 91), herpes simplex virus (92), mycobacterium tuberculosis (93) and parasites (94). Furthermore, microbial and/or antigen-specific memory $CD4^+$ and $CD8^+$ T_{RM} cells produce vast amount of effector cytokines in response to microbes and antigens (95–97) and $CD4^+$ and $CD8^+$ T_{RM} cells can populate and persist in multiple tissue sites long after the microbe or the antigen has been neutralized (98, 99). In the skin, $CD8^+$ T_{RM} can be generated following an infection (92, 100, 101) and $CD4^+$ IL-17-producing T_{RM} cells were identified in the skin of the mice when they were infected by *C. albicans* (part of skin mycobiome) (102). Besides, another study showed that laboratory SPF (specific-pathogen free) mice had lower non-circulating T cells in the skin and

other tissues compared to pet store mice (103). In terms of T-cell memory, SPF-raised mice have a similar adaptive immunity like newborn humans and pet store mice show the profile of memory T cells, similarly observed in adult humans (104). Several studies show a compartmentalization of microbe-specific memory T cells. When humans were injected intradermally with purified protein-derivative from *M. tuberculosis*, antigen-specific T cells were observed only in the skin but not in the blood (105). HSV2 specific $CD8^+$ T cells were found in genital skin but not at other body sites (106). Variability within the skin microbiome (16) could be a reason for compartmentalization of T_{RM}. Skin T_{RM} persists for long periods of time and are exposed to the microbiome and microbial antigens from the skin during their lifetime. Microbial-specific responses could be a part of the healthy immune balance between the skin microbiome and host immune system and further provide reinforced local immunity. Very interestingly a recent study demonstrated that non-invasive *S. epidermidis* allows specific $CD8^+$ T_{RM} establishment through non-conventional MHC-Ib H2-M3 peptide presentation. Those H2-M3 restricted $CD8^+$ T_{RM} were shown to play an important role in tissue repair and wound healing (107).

PERSPECTIVE

Skin microbiome and T_{RM} reside in the upper layers of the skin. Both UV-A and UV-B radiation can penetrate those upper layers (only UV-A particularly reaches the dermis) and imminently impact all the microbes and immune cells (**Figure 1**).

Does UV-R Directly Shape the Persistence, Phenotype, Specificity and Function of Skin T_{RM}?

UV-R is known to induce production of various cytokines in the skin such as TNF-α (108) or IL-33 (109–111) which are known to be involved in maintaining the phenotype of T_{RM} (52, 64, 112). Besides, a study published in 2016 (62) linked UV-B exposure and T_{RM} retention. Authors demonstrated that UV-B exposure decreased αvβ6 and αvβ8 integrins expression by keratinocytes. Those integrins were required for active TGF-β production which then maintained CD103 expression on T_{RM} allowing their retention in the skin long time after a lymphocytic choriomeningitis viral infection. Hence, the ability of UV-R (notably UV-B) to dose-dependently influence the retention and phenotype of skin T_{RM} by modulating the cutaneous cytokine environment (**Figure 2**), certainly may at least contribute to the efficacy of suberythemal phototherapy, which has been used for decades to improve pathologies such as psoriasis, atopic dermatitis and other inflammatory diseases (113–116). However, beyond cytokines, it is also possible that T_{RM} persistence depends on TCR-specific signals. The discovery of commensal-specific T_{RM} in the gastrointestinal tract of mice (88) implies that there may be a large number of commensal-specific T_{RM} residing in the skin as well, in addition to γδ T cells, innate lymphoid cells and pathogen-specific T_{RM}. Moreover, the skin microbiome is constantly changing within the individual throughout lifetime (117) and contributes to skin T_{RM} diversity and function (107). Interestingly, UV-R is known to influence the skin microbiome landscape (8, 18, 19, 31). UV may in a dose dependent fashion affect skin microbiome and may shape the repertoire diversity of effector or regulatory T_{RM}. Important remaining questions are the contribution of T_{RM} to the local immune response against (i) non-specific, commensal microbes which could invade the skin upon a skin barrier damage and (ii) invading pathogenic microbes. The first question queries upon their role in chronic pathologies such as psoriasis, atopic dermatitis or PLE. The second question concerns the capacity of T_{RM} to provide a heterologous protection against diverse infections (118) (**Figure 2**).

Does UV-R Alter Skin Barrier Function, Further Activating Microbe-Specific Skin T_{RM} and Causing Chronic Inflammation?

Commensal microbes are known to improve innate and adaptive responses by producing small molecules which act as mediators between the host and microbes (119). Recently it has been reported that commensal skin microbiome can modulate gene expression of various cytokines, TLRs and AMPs in total skin cells (120). In the skin *Staphylococcus aureus* is known to promote skin inflammation by producing phenol-soluble modulins (PSMs) (121) which can stimulate IL-1-type (IL-36α and IL-1 α) cytokine production (122) and IL-17 from dermal γδ T cells (123). Moreover, *S. aureus* secretes proteases which are involved in skin barrier damage, promoting bacterial penetration into the skin which could ultimately generate *S. aureus*-specific T_{RM} cells. A robust accumulation of commensal-specific T cells under defined conditions may lead to worsening pathogenic conditions such as psoriasis (124, 125). Psoriasis and AD are intriguing examples of possible T_{RM} interplay with commensal microbes. An inflammatory environment exists in these chronic diseases which may lead to severe barrier disruptions through the patient's life. This could eventually lead commensal microbes to penetrate the skin, produce microbial-antigens, and finally lead to specific T_{RM} recruitment and establishment at the inflammatory site. In this context, both allergen-specific T_{RM} and commensal microbe-specific T_{RM} are in place. Whether commensal-specific T_{RM} cells portray a regulatory role or participate in the inflammatory loop is not known. Commensal-specific T_{RM} may also play a role in PLE, an inflammatory skin condition in which itchy skin lesions of diverse morphology occur when the skin is exposed to sunlight. In this disease microbes residing on upper layers may be driven to induce the production of AMPs and express commensal associate molecular patterns (126) which could play a role in pathophysiology of the disease. Furthermore, the capacity of UV-R to cause a barrier defect (127) may contribute to this phenomenon. Patients developing PLE may have skin inhabiting or newly generated commensal-specific T_{RM} that get activated. An inflammatory microenvironment may lead to changes in microbial landscape, further increase specific T_{RM} activation and booster the inflammatory loop.

CONCLUSION

The specificity of adaptive immune system is complexly linked to the establishment and the persistence of the T_{RM} which recognize previously encountered antigen via specific T cell receptors (TCRs). These specific T_{RM} are generated and kept as a pool of heterogenous population with respect to the numerous microbes and microbe-associated antigens that they encounter during the lifetime of individual. With recent discoveries about potential functions of skin microbiome to educate and modulate host-immune responses, it is important to identify how these microbes influence the skin T_{RM}. Specifically targeting those T_{RM}, directly or via microbiome may allow to develop novel treatment strategies, acting like or even better than phototherapy, but with an improved risk-safety profile.

AUTHOR CONTRIBUTIONS

VP and LL: conceived the ideas and drafted the manuscript; VP: drafted the figures; J-FN, MV, and PW: corrected and contributed to the draft. All authors revised and approved the final version of the manuscript.

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Pathogenesis of Skin Carcinomas and a Stem Cell as Focal Origin

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UV radiation in sunlight has long been recognized as the main exogenous cause of skin carcinomas. We present a brief historical perspective on the progress in understanding the pathogenesis of skin carcinomas, and recent advances. Sun-exposed skin carries numerous UV-related mutations, and skin carcinomas rank among the tumors with the highest mutational loads. In this multitude of mutations only a few are crucial in driving the tumor. Some are known from hereditary (skin) cancer syndromes and other recurrent ones have been validated in transgenic mice. Considering the continuous renewal of the epidermis, the question arises whether the lifelong residing stem cells are the main targets in skin carcinogenesis, a multistep process that would require ample time to evolve. Therefore, classic quiescent stem cells have been studied as potential tumor-initiating cells, as well as more recently discovered actively dividing stem cells (either Lgr5+ or Lgr6+). Interesting differences have emerged between experimental UV and two-stage chemical carcinogenesis, e.g., the latter appears to originate from follicular stem cells, in contrast to the former.

Keywords: skin carcinoma, UV radiation, stem cells, quiescent, Lgr5, Lgr6

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INTRODUCTION

Skin cancers had already been linked to excessive sun exposure in the nineteenth century, specifically skin carcinomas were found predominantly in people with outdoor jobs. Genotoxicity, mutagenesis, and carcinogenesis by UV radiation, as present in sunlight, were experimentally established in the early decades of the twentieth century. Before the 2nd World War spectral analyses showed that DNA was the target of UV radiation for cell death and mutations (1, 2): i.e., well before Watson and Crick published the correct model of the structure of DNA, explaining how genes made up of DNA carried the genetic code which could be straightforwardly copied for daughter cells. Miscopies would introduce mutations. Consequently, replication of damaged DNA, hampering correct copying, for cell division was identified as the most prominent cause of mutagenesis. Carcinogenesis is considered to evolve primarily as a "multi-hit" process in which mutations accumulate in cells until a combination of mutations (and possibly other genetic defects and epigenetic modulatory effects) emerges which drive a cell to malignancy. As such a cell destined for malignancy requires time and cell divisions to transform, the most likely candidates would appear to be adult stem cells that constitute the very basis of tissue renewal. This premise was evidenced by a correlation that Tomasetti and Vogelstein (3) found between rate of stem cell division in various tissues and the risk of cancer. This led them to the controversial statement that most cancers are "bad luck" arising from an inherent risk of mutation in cell division. UV

irradiation is known to cause epidermal hyperproliferation and hyperplasia. This would increase the UV-related risk of carcinomas originating from the epidermis (4), in addition to the risk derived from the genotoxicity of UV radiation.

HUMAN SKIN CARCINOMAS AND SUN EXPOSURE

The skin is an evident frontier of the body in interactions with its environment. UV radiation in sunlight poses a recurrent (geno-) toxic challenge to skin, and like all life dwelling on the Earth's surface, it has powerful defense mechanisms, among which very importantly Nucleotide Excision Repair (NER) to maintain the integrity of the genome. A defect in NER increases the risk of skin cancer dramatically to the point that 50% of patients with Xeroderma pigmentosum succumb to multiple skin cancers before the age of 30 (5). NER eliminates the dominant UV-induced DNA damage (cyclobutane pyrimidine dimers, CPDs, and 6–4 photoproducts, 6–4 PPs) by a "cut-and-paste" action: cut out an oligo with the damage and fill in the gap using the complementary strand. As this UV-induced DNA damage occurs predominantly at neighboring pyrimidines in a DNA strand, the resulting mutations (mainly C > T) are located at dipyrimidine sites, and referred to as UV signature mutations. Strikingly, mutations in the *P53* tumorsuppressor gene of skin carcinomas show predominantly this UV signature (6). Microscopic clusters of cells (clones) overexpressing mutant P53 are present in chronically exposed skin, and presumed to be potential precursors of skin carcinomas (7). More recently, deep sequencing of 74 cancer-related genes (incl. *P53*) has shown that sun-exposed skin (from eye lid resections) is full of mutations (2–6/Mb), with a majority of UV signature mutations and an estimated average of 140 small clones/cm^2 with a mutation in one of these 74 genes (8). Strikingly, another recent study found SCC-related mutations to be restricted to P53-overexpressing cell clusters (9).

The authors (8) noticed that the sun-exposed skin appeared clinically normal despite the high mutation load, and that the clones remained restricted in size. Apparently, the skin is inherently able to cope with a multitude of mutated clones. In experiments with Wnt-activated clones, it was shown that in signal exchange the normal cells were stimulated to outcompete the mutated cells (10). Much earlier, it was reported that low grade malignant keratinocytes were kept in check to contribute to epidermal homeostasis by surrounding normal keratinocytes (11). Hence, the outgrowth of cells into a tumor would appear to require the collapse of growth control by surrounding normal cells.

Considering the high mutation load in sun-exposed skin, it is no surprise that skin carcinomas belong to the absolute top of cancers with high mutation loads (10,000–100,000 per cell). Mutation load was found to be proportional to the immunogenicity of a tumor (12) and consequently proportional to the success of immunotherapy by check-point inhibition (13). In immunosuppressed organ transplant recipients the risk of skin cancer is raised, most dramatically the risk of squamous cell carcinoma, SCC (14) which correlated with preceding cutaneous HPV infections (15).

DRIVER MUTATIONS

With an overwhelming load of mutations it would appear impossible to separate the driver mutations from passenger mutations. However, recurrent mutations within this multitude could be considered drivers, and earlier on, potential drivers were identified from syndromes with an inherited pre-disposition to develop cancers. A textbook example of the latter is the Gorlin syndrome (Basal Cell Nevus Syndrome, BCNS) where mutations in the tumorsuppressor *PTCH* gene predisposes to activation of the Hedgehog pathway (e.g., by loss of the wt allele by UV radiation) and subsequent formation of multiple basal cell carcinomas, BCCs (16). Also, most sporadic BCCs turned out to be driven by an activated Hedgehog pathway commonly involving mutations in *PTCH* or *SMO* (17). Activation of the Hedgehog pathway or ectopic expression of its downstream transcription factor, Gli1, in mouse skin gives rise to BCCs (18, 19).

In malignant progression of SCCs the RAS pathway was found to be activated (20, 21), however, apparently without any relevant recurrent mutations, notably rarely mutations in (*Ha-*)*RAS* genes (22). Next to a predominance of UV signature mutations in *P53,* nearly all SCCs were found to bear such mutations in one or more of the *NOTCH (1–4)* genes (23). *NOTCH1* mutations were already present in early stages of SCC development (24). Transgenic mice in which epidermal Notch signaling was blocked developed SCCs (25).

WHAT CELL DRIVES THE OUTGROWTH OF HUMAN SKIN CARCINOMA?

It is notoriously difficult to propagate skin carcinoma cells *in vitro* and establish cell lines. Our group could only maintain fresh SCCs intact as explants (26). Others were successful in culturing SCC cells on fibroblasts (3T3) as feeder layers (27). In contrast to normal fibroblast, the cancer-associated fibroblasts (CAFs) appear to harbor a special class of fibroblasts facilitating invasion of SCC into the dermis (28). SCCs show a clear heterogeneity with differentiated keratinocytes (around keratin "pearls," horny layer-like deposits) enclosed by germinative basal cell layers of keratinocytes bordering and infiltrating the stroma. Like in normal epidermis, the stem cells that drive SCC, the tumor-initiating cells (TICs), are logically expected to reside in the germinative compartment of the tumor. CD133 (prominin-1) is a tumor stem cell marker (e.g., in lung cancer), and not detectable in normal epidermal keratinocytes (proteinatlas.com). But some cells in germinative outer rim of SCCs are CD133-positive, about 1% of the tumor cells (27). Transferring as few as 100–1,000 of these CD133+ cells in combination with a million of human fibroblasts in matrigel into a pre-created subcutaneous space resulted in a 50% chance to spawn a new SCC in immune compromised mice (not capable of rejecting the human SCC). Evidently, the human SCC TICs needed the

microenvironment of human fibroblast to support the outgrowth (generating appropriate CAFs?). It is not clear whether or how the CD133+ cells are related to stem cells of the normal human epidermis.

Similar results using the subcutaneous transplant assay have been obtained with CD200+ cells from BCCs, about 1–2% of the tumor cells (29). In contrast to CD133, CD200+ cells are present in normal skin: specifically in hair follicles in the region (the bulge) where stem cells reside in mice. However, this does not necessarily imply that the BCCs originate from these cells in hair follicles [although tracing mitochondrial DNA mutations by COX-deficiency would support this (30)]. Activation of the Hedgehog pathway and further transformation could conceivably lead to CD200 expression in the TICs. As monotherapies with SMO antagonists (e.g., vismodegib) inhibiting the Hedgehog pathway are not curative, the authors suggest to target the CD200+ cells instead for a permanent elimination of the tumor.

HISTORICAL PRELUDE TO EXPERIMENTAL SKIN CARCINOGENESIS

Present day research on experimental skin carcinogenesis employs two basic mouse models, chemically or UV driven, which stem from historical observations on skin cancer in man. First of all, the surgeon Sir Percival Pott (a founder of orthopedics) reported in 1775 on the frequent occurrence of scrotal cancer (SCCs) among chimney sweeps in London, and recognized soot (coal tar) as the evident culprit (31). And secondly, skin carcinomas were linked to sun exposure at end of the nineteenth century. In Hamburg the dermatologist Unna (32) stated in his book on skin diseases that degenerative changes in the sun-exposed skin of sailors ("Seemanshaut") were associated with skin carcinomas. In Bordeaux Dubreuilh (33) noticed that vineyard workers contracted remarkably more skin carcinomas than people living in the city. Further detailed observations on body locations of the carcinomas indicated that they were most likely caused by sunlight.

CHEMICAL CARCINOGENESIS

Just before the First World War, the first experimental proof of tumor formation by coal tar was provided by the Japanese pathologist prof Yamagiwa. It was done in rabbits by repeated applications of coal tar to the ears. Yamagiwa had visited the Virchow Institute in Berlin where he learned about Virchow's irritation theory ("Reiztheorie") of carcinogenesis (34). The experiment was modified in mice to include "cocarcinogens" (35), such as the "irritant" croton oil which "promoted" tumor outgrowth (reminiscent of the "Reiztheorie"). From these early experiments the standard classic two-stage protocol evolved in which a single genotoxic challenge with, for example, coal tar [or one of its ingredients like benzo(a)pyrene] irreversibly "initiated" tumors after which tumor development was "promoted" by a regimen of repeated applications of an "irritant" like croton oil (or its active ingredient 12-O-tetra- decanoyl-phorbol-13-acetate, TPA, activating PKC) (36). Tumor promotion was reversible in that tumors would not develop or regressed up on early termination of this regimen. This protocol yielded exophytically growing, wart-like, benign tumors (papillomas), and at a later stage some SCCs. *Ha-ras* mutations were commonly present in these tumors, notably already at the earliest tumor stages in hyperplastic foci in hair follicles (37). And even earlier, *Ha-ras* mutations could be detected by nested PCR from expanding clones in the in normal looking skin that had been subjected to the two-stage protocol (38). In contrast to *Ha-ras*, *p53* mutations occurred late in tumor progression and were linked to malignant conversion (39). Over a period of 80 years chemical carcinogenesis took central stage because of experimental convenience and because of its versatility in analysing the biology of carcinogenesis and in characterizing (anti-) carcinogenic substances and their interactions.

UV CARCINOGENESIS

Experimental proof of tumor induction by UV radiation was first published in 1928 by Findlay (40) who had chronically irradiated depilated albino mice for 8 months with a quartz mercury lamp. Interestingly, he also found that painting the animals with coal tar before irradiation speeded up the development of tumors (<3 months). Next, the prolific Brazilian professor of pathology Angel Roffo—who also pioneered in showing benzpyrene from tabacco to be carcinogenic—showed in the 1930s that the UV part in sunlight blocked by window glass ("UVB") to be carcinogenic on rats (41, 42). The exact wavelength dependence (action spectrum) was determined much later in the 1990s for SCCs in hairless mice (43). The early experiments were done on the ears (and tails), or shaven backs of haired mice, but in the 1960s the more convenient and sensitive hairless mouse model was introduced which has become a standard in experimental UV carcinogenesis (44). In contrast to hairless mice, haired mice were reported to developed fibrosarcomas next to SCCs under chronic UV exposure (with substantially higher UV dosages than used on hairless mice). However, this was corrected by showing that the tumors were keratinocyte-derived (i.e., exclusively epidermal) and ranged from well differentiated to spindle cell carcinomas (45). The tumor progression in hairless mice was very similar to that in humans starting with endophytically growing actinic keratosis as benign precursor lesions (majority of tumors <2 mm across) of which a fraction progressed to malignant SCCs (majority > 3 mm) (44), and with a majority throughout bearing UV signature mutations in the tumorsuppressor *p53* (46); even before tumors appeared, microscopic clusters overexpressing mutant-p53 could be detected in the chronically sub-sunburn UV-exposed skin (47). *Ras* mutations were virtually lacking in the tumors: only 1 tumor with a *Ki-ras* mutation out of 32 tumors, none with a *Ha-ras* mutation (48). Only with a NER defect, in XPA mice, did *Ha-ras* mutations occur in UV-induced tumors which notably were benign papillomas as found in chemocarcinogenesis (49). The repair defect impaired removal of CPDs from the transcribed strand of *Ha-ras*. This introduced

novel mutational targets for UV radiation corresponding with the oncogenic *Ha-ras* mutations. Overall, the mutational spectrum of UV SCCs in hairless mice resembled that of human SCCs, including Notch 1–4 mutations (50). In the 1970s it was discovered that UV-induced tumors were antigenic and that UV irradiation raised a specific immune tolerance toward these tumors (51). Recently, cutaneous papilloma virus infection was shown to enhance UV carcinogenesis (52). In all, experimental UV carcinogenesis shows striking parallels with human SCCs supporting the validity of the model.

STEM CELLS

A remarkable difference between chemical and UV carcinogenesis appears to be the origin of the SCCs. After initiation, abrasion of the interfollicular (IF) epidermis did not affect development of chemo-SCCs, indicating that they originated from the hair follicles (53). In contrast, our group showed that apoptotic elimination of the IF basal layer by a single UV overdose nullified the UV carcinogenic regimen up to that point and carcinogenesis had to restart afterwards, indicating that UV-SCCs originated from the IF epidermis (54).

The observation that the interval between tumor initiation and promotion could be extended to months demonstrated that the initiated cells were not shed in epidermal turnover and were therefore likely to be stem cells. This was confirmed by radioactive tracing of the initiating substance, benz(a)pyrene, which was retained in hair follicles and interfollicular epidermis in label-retaining cells, i.e., in quiescent stem cells (55). CD34+ cells located in the bulge of hair follicles were found to harbor such quiescent cells (56) and they were identified as tumor stem cells, or tumor-initiating cells (TICs), in chemically induced skin tumors (57). We similarly found that IF quiescent cells retaining CPDs from a low level UV regimen were linked to the development of non-regressing *in situ* carcinomas after TPA tumor promotion (58). There is, however, no established reliable protein marker for IF quiescent cells (resting or activated) by which to identify these cells in a tumor mass; putative stem cell markers (Wif-1, Lrig1, Dll1) did not label the CPD-retaining quiescent cells. Our group earlier identified Mts24/Plet1 as a stem cell marker (59) but later found this marker expressed in differentiated cells after UV exposure (**Figures 1a,b**) and in papillomas (**Figures 1c,d**) but absent in SCCs (not shown) (60). This demonstrated that a stem cell marker in homeostasis need not be one under (UV) stress, in hyperplasia or in tumors.

Recently, a new class of proliferating stem cells (either Lgr5+ or Lgr6+) was studied as possible TICs in chemical and UV carcinogenesis; this was done by "lineage tracing" to identify the progeny of these stem cells in tumors. Lgr5+ cells and progeny were not detected in either chemically or UV-induced tumors (61, 62). Our group could not detect any appreciable presence of Lgr6+ cells in tumors and only some sporadic remnants of progeny deep into the differentiated compartments, i.e., no indication that Lgr6+ cells were TICs or drove tumor growth (63). In contrast, Huang et al. (62) reported the presence of

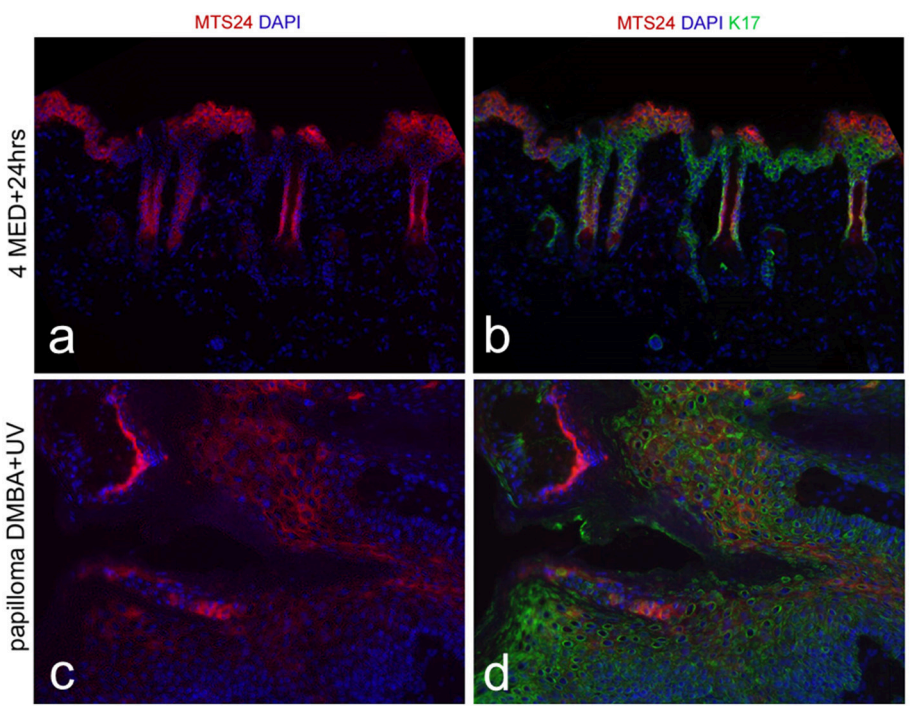

FIGURE 1 | Mts24 fluorescence in red **(a,c)** in hairless mouse skin, combined with K17 in green **(b,d)**; **(a,b)** 24 h after high UV dose (4x threshold dose for a sunburn reaction) with Mts24+ cells high up in de epidermis in differentiated cell layers; **(c,d)** papilloma after neonatal DMBA (dimethylbenz [α]anthracene) followed by chronic UV exposure with Mts24+ cells throughout the tumor mostly differentiated cells (60), reproduced with permission.

Lgr6+ cells in chemically induced tumors in a different mouse strain than we used, and with a different protocol for lineage tracing. However, these Lgr6+ cells did not exclusively reside in the germinative compartment of the tumors, showed a lack of expression of K14 (marker of germinative basal cells) and some even appeared flattened out in terminal differentiation. Apparently Lgr6 was no longer a marker of stem cells in these tumors (reminiscent of what we found with Mts24/Plet1). Intriguingly, Huang et al. (62) concluded from experiments with Lgr6 knockout mice that Lgr6 in normal epidermis functioned as a tumor suppressor. Interestingly in this respect, we found that Lgr6+ cells and progeny were lost from IF epidermis under chronic UV exposure long before the occurrence of SCCs; in contrast, a TPA regimen caused a clear expansion of progeny in the IF epidermis (63).

CONCLUSION

From the present vantage point, UV carcinogenesis in mice appears to emulate SCCs in humans better than two-stage chemical carcinogenesis. And the quiescent stem cells appear to be the most likely target cells from which SCCs arise, either from quiescent cells in hair follicles in chemocarcinogenesis or quiescent cells in the IF epidermis in UV carcinogenesis. Future research should be directed toward identifying the latter cells by reliable protein markers, which may subsequently serve to develop well targeted interventions to prevent or cure cutaneous SCCs.

As there is no robust mouse model available for the *de novo* induction of BCCs by exogenous agents, identification of the primary target cells requires further research.

AUTHOR CONTRIBUTIONS

FdG (biophysicist/photobiologist): outline of review, wrote draft, finalized text, and references. CT (molecular biologist): outline of review, brought in and checked molecular aspects, filled in some gaps, edited draft versions.

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Sun Exposure and Melanoma, Certainties and Weaknesses of the Present Knowledge

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Sun exposure is the main risk factor for cutaneous malignant melanoma (CMM). However, the UV-related pathogenetic mechanisms leading to CMM are far to be fully elucidated. In this paper we will focus on what we still don't fully know about the relationship between UVR and CMM. In particular, we will discuss: the action spectrum of human CMM, how different modalities of exposure (continuous/ intermittent; erythemal/ suberythemal) relate to different CMM variants, the preferential UVR induced DNA mutations observed in different CMM variants, the role of UV-related and UV-unrelated genetic damages in the same melanoma cells. Moreover, we will debate the importance of UVA induced oxidative and anaerobic damages to DNA and other cell structures and the role of melanins, of modulation of innate and acquired immunity, of vitamin D and of chronic exposure to phototoxic drugs and other xenobiotics. A better understanding of these issues will help developing more effective preventative strategies and new therapeutic approaches.

Keywords: melanoma, sun exposure, vitamin D, UVA, UVB

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INTRODUCTION

It is widely accepted that ultraviolet light radiation (UVR) is the major—but not the only—risk factor for the development of cutaneous malignant melanoma (CMM) (1). It is thought that genotoxic, inflammatory, and immunosuppressive properties of UVR contribute together to initiation, progression, and metastasis of CMM. However, several important mechanistic details regarding how sunlight causes CMM remain to be fully elucidated. As a consequence, we still cannot provide fully effective preventative behavioral strategies. In the present paper, we will focus on the main weaknesses of the present understanding of UVR-CMM relationships.

MODALITY OF UVR EXPOSURE AND CMM VARIANTS

Cutaneous malignant melanoma (CMM) is not a single tumor entity with a homogeneous profile of risk factors and prognosis. Consequently we recognize a few variants. It is completely unclear why different modalities of UVR exposure (erythemal/suberythemal doses; chronic/intermittent exposures) induce different molecular damages in the same cell population (2) and why these different molecular damages lead to different clinical CMM variants. For example, we do not know why chronic lifetime sun damage, seen in elderly people and outdoor workers, is related to the specific pattern of DNA mutations characteristic of Lentigo Maligna Melanoma (LMM) (3), while

sunburns do not seem to be a significant risk factor for this CMM variant (4). In contrast, Superficial Spreading Melanoma (SSM) and Nodular Melanoma (NM), that have a different spectrum of UV related DNA mutations, usually develop on intermittently exposed healthy skin of younger subjects (3) and a history of sunburns (particularly during childhood) was found to double the risk (5).

WAVEBAND DEPENDENCY OF GENOTOXIC DAMAGES AND CMM

The most relevant cromophore for skin carcinogenesis is DNA. Its absorption peak is in the UVB region. The different types of UV-DNA photoproducts and their waveband- dependency are summarized in **Figure 1**. Cyclo-butane pyrimidine dimers (CPD) (T<>T, C<>C, C<>T, and T<>C)-and 6–4 photoproducts (6–4PP) are the most frequent. Until few years ago, it was assumed that UVB biological effects were mainly caused by oxygen-independent reactions, whereas UVA reactions were considered exclusively oxygen dependent. As a consequence, the terms "UVB effects" and "UVA effects" were used as synonyms for anaerobic (synonyms: direct, anoxic or type 1) and aerobic (synonyms: indirect, oxidative or type 2) effects, respectively. However, it was demonstrated that UVA induces DNA type 1 damages as well (6). Indeed, UVA seems able to produce oxidative DNA damage directly or after the oxidative sensitization of not yet identified endogenous photosensitizers (7). Concerning DNA damages, it is therefore clear that the sharp distinction between UVB and UVA, should be reconsidered, because C -> T transitions and CC -> TT tandem mutations (8) are no more to be considered as only UVB damages. Therefore, the term "UVB signatures" should be avoided because potentially misleading.

UVA genotoxic activity is about 1,000 times weaker than UVB's one if considered on a per photon basis (6–8). However, its importance is partially compensated by the UVA environmental irradiance that is about 20–40 times higher, depending on some factors, including time of day, season, latitude and altitude (9). In addition, UVA irradiation is even higher when UV exposure happens through a window glass or in sunbeds. Also, the application of non-broad-spectrum sunscreens, not able to filter UVA as much as UVB, can cause UVA over- exposure. In the same way, aerobic damages to DNA, leading to the formation of 8-oxo-7,8-dihydro-2′deoxyguanosine (8oxo-dG) and consequent reparation with G→ T transversions and G→ A transitions, cannot be considered "UVA signatures" because UVB is able to produce aerobic damages as well (6, 8).

Other mutations, induced by both UVA and UVB, are DNA-protein cross-links and single and double strand breaks, but their role in CMM development remains to be clarified (6, 8). Anaerobic UV DNA damages are repaired by the nucleotide excision repair (NER) system, while oxidative DNA damages are repaired by the base excision repair (BER) system. It is interesting to note that the mutation rate per DNA photoproduct is higher with UVA: in fact UVA damages are not followed by as many protective, anti-mutagenic and reparative responses as UVB damages are (10).

Anaerobic and aerobic photoproducts of DNA, together with other biomolecules, induce a cascade of pro-inflammatory signals and suppress pro apoptotic pathways (**Figure 2**). However, the respective relevance and interplay of UVA- and UVB- activities are still largely unknown. Finally, we emphasize how important it is to extrapolate in a very careful and critical manner the results of experimental studies about photo-genotoxicity made with cell cultures: during "*in-vivo*" UV exposure, while UVA has a deeper penetration, only a small fraction of the incident UVB radiation reaches the level of the dermal-epidermal junction of human skin, where melanocytes are located. We should also be careful with findings of studies on animals, because penetration into human skin may be different.

UVR AND GENE MUTATIONS

Gene mutations found in CMM cells are more frequent at selected loci. Aiming to understand their possible diagnostic and prognostic meaning, they have been divided in two main groups: mutations providing no selective advantage to the tumor growth (that occur stochastically during cancer development), and genomic alterations that have a role in cancer development or in the determination of cancer phenotype. In order to assess a correlation between sun exposure and mutations at hot-spots within promoters, the detection of canonical UV signatures (C to T and CC to TT mutations) is mandatory.

UVR-induced mutations are frequently found in the CDKN2A gene in all CMM variants. In humans, this gene encodes for the tumor suppressor proteins p16 and p14ARF (11).

N-RAS is the most frequently affected RAS family member in CMM. Both anaerobic and oxidative, as well as non-UV related, damages have been detected on this gene (12). These mutations can lead to the production of a permanently activated RAS protein, causing the consequent activation of phosphatidylinositol 3′ kinase and mitogen-activated protein kinase (MAPK) pathways. The constant activation of these two pathways leads to unintended and overactive signaling for cell growth, differentiation and survival even in the absence of incoming signals (13). If the frequency of NRAS mutations in LMM, in comparison to SSM and NM, is higher (14, 15) or not (16, 17) is still debated.

The BRAF gene encodes for a serine/threonine kinase that plays a key role in the MAPK signaling pathway. BRAF mutation is more often associated with SSM and NM and it is particularly common in younger patients (18–22). KIT mutations are often found in acral lentiginous melanoma (ALM) and mucosal melanoma (MuM), less frequently in LMM, and rarely in SSM and NM (23). The gene encoding for the pro-apoptotic p53 is another frequent target of UVB damage. Mutated p53 is often observed in melanoma metastases (24), while it is less frequent in primary CMM. This clearly indicates a role for UVR in CMM progression (25, 26). The Nucleotide Excision Repair (NER), and in particular the global genome repair (GGR) damage recognition sub-group, may also be damaged by UVR in melanoma cells, leading to a defective DNA repair response (27, 28).

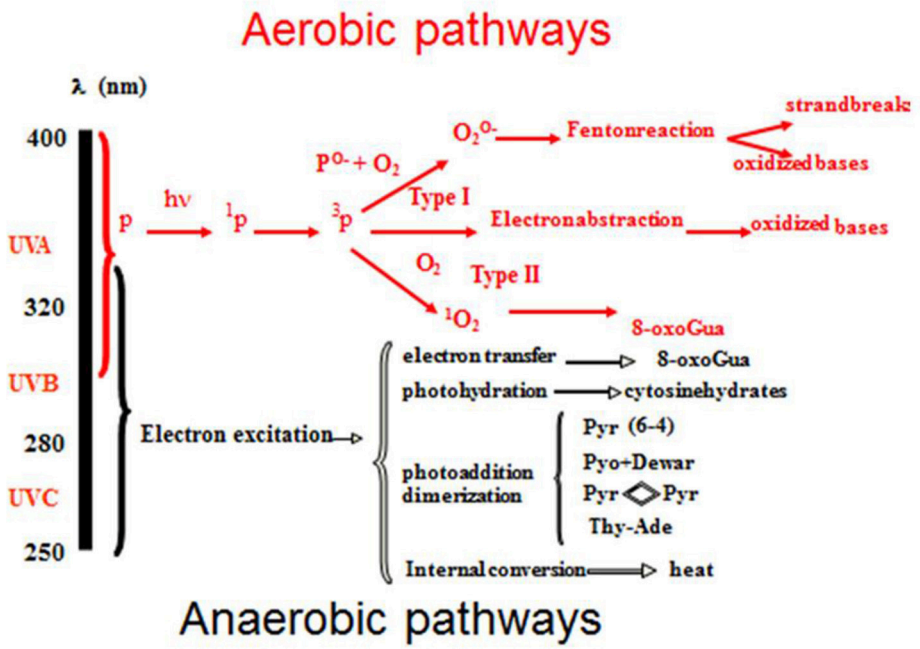


FIGURE 1 | UV- induced DNA damages and their waveband dependency.

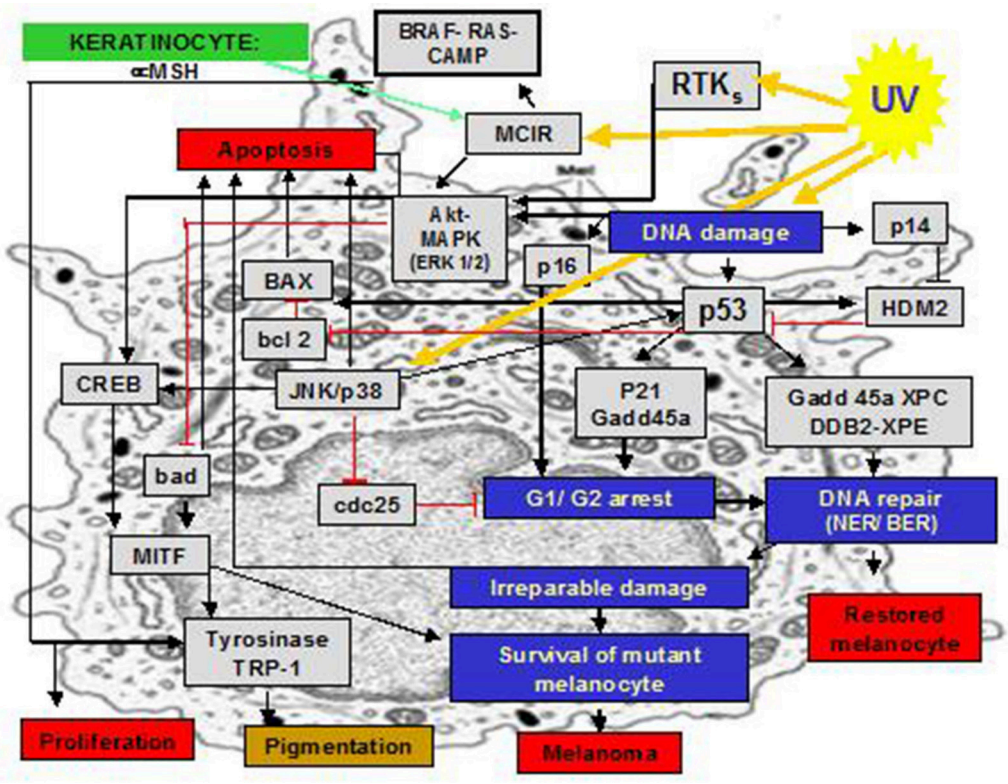


FIGURE 2 | Selected inflammatory molecular pathways that are triggered by UVR in melanocytes.

Therefore, we can conclude that clinical variants of CMM are differently associated to different driver mutations (**Figure 3**). However, the biological mechanism for which selected DNA mutations drive the mutated melanocyte to a specific clinical variant of CMM is unknown. Furthermore, a significant correlation between clinical outcome and genomic damages is yet to be found (29).

UV-RELATED AND NON UV-RELATED DNA DAMAGES IN MELANOMA CELLS

Even if the great majority of BRAF, RAS, and NF1 mutations harbored UV signatures (26, 30) they also showed a high burden of non-UV related mutations (31). This suggests that UV has a role in melanoma pathogenesis, but UV-unrelated mutations can play a role as well. The pathogenic contribution of these UV-independent mutations is still to be clarified. In addition, analysis of whole-genome sequences reveals different carcinogenic processes across the CMM subtypes, some unrelated to sun exposure, and extends potential involvement of the non-coding genome in its pathogenesis (31).

UVR, MELANOMA AND MELANINS

The protective role of eumelanin is suggested by the evidence that people with dark skin are less prone to develop CMM. Indeed, eumelanin has UV-filtering properties. However, experimental findings have shown that the relationship between CMM and melanins is more complex. The action spectrum of human CMM is unknown but, in the 90 s, Setlow et al. demonstrated that UVA and UVB wavebands have similar pathogenetic activity for CMM in the xiphophorus fish model. Furthermore, they demonstrated that melanin-photosensitized radical production is the major causative step of CMM of this fish (32). Recent experimental work in transgenic mice, confirmed that UVA dependent eumelanin's pro-oxidative activity has a significant pathogenetic role for CMM (33). In addition, unlike UVB, that initiates CMM in a "pigment-independent" manner through direct DNA damage, UVA was found to require the presence of melanin (33). Furthermore, it is worrying to know that, in a preliminary study, it was found that the majority of UVA-induced CPDs in melanocytes are generated after more than 3 h from exposure (34). These "dark CPDs" arise when UVA-induced reactive oxygen and nitrogen species combine to excite melanin that induces CPDs by energy transfer to DNA, in a radiation-independent manner (34). Studies on albino African people provide more evidence that melanin is important for CMM development. The incidence of CMM in this population is low while they still early produce several non melanoma skin cancers (NMSCs).

The highest risk of CMM belongs to people with a 'red hair/fair skin' phenotype, who synthesize a great amount of phaeomelanin. People with homozygote and heterozygote red hair MC1R variants have eumelanin/ pheomelanin ratio of 1.46 and 4.44 respectively, while wild types have 5.81. Unlike eumelanin, phaeomelanin has poorer protective activity against UVR and greater oxidative potential, both in the dark and after UV exposure (35). Phaeomelanin synthesis is regulated by the MC1R gene. Its variants could play a role in CMM development, also via non-pigmentary pathways (36), including a defective control of α- melanocortin (α-MSH)-mediated DNA repair (37, 38), repair of oxidative DNA damage (39), Nucleotide excision repair system and PTEN- dependent pro-apoptotic pathway (40).

Beside red hair subjects, specific MC1R variants may be also found in people with dark hair. These people have an increased risk to develop CMM. Finally, MC1R regulates the expression of the transcription factor MITF that, in addition to pigment biosynthesis enzymes, regulates genes that control DNA repair (APEX nuclease1) (41), cell cycle (CDKN2A, CDK2) (42, 43), apoptosis (BCL2) (44), and invasion (DIA1) (45).

UVR, PHOTOTOXIC DRUGS AND MELANOMA

Experimental and clinical findings suggest that drugs (e.g., azathioprine, vemurafenib, fluoroquinolone antibiotics, propionic acid derivative NSAIDs and voriconazole) can favor melanomagenesis following activation by repeated sub-erythemal UVA exposure (46–48). Also, high levels of folic acid are claimed to have a genotoxic potential because of their pro-oxidative activity that can be further enhanced by pre-treatment with methotrexate (49). The relevance of these findings in the general population is however still to be elucidated.

UVR INDUCED METABOLIC CHANGES IN MELANOMA

UVA radiation can play a very important role at an early stage of metastasis through mechanisms that are not directly depending on DNA damage. After repetitive exposures to low doses of UVA, glycolysis and lactate production are increased (Warburg effect) (50). This effect persists for at least 5 days after the last UV exposure and is associated with an up-regulation of several matrix metalloproteinases (MMP2, MMP3, MMP9, MMP13, MMP15), with a consequent increment of melanoma invasiveness (51, 52). Warburg effect increases the speed of tumoral cell mitosis too, because part of the glycolysis-derived pyruvate can be used for anabolic pathways (amino acid or fatty acid synthesis) (53).

PHOTOIMMUNOLOGY

CMM is a potentially highly immunogenic tumor due to its multiple auto-antigens (54). However, UV exposure produces a partial loss of immuno-surveillance by decreasing the number and functionality of antigen presenting cells (both Langerhans and dendritic cells) (55, 56). This leads to a shift of the immune response from Th1 to Th2 (57) and the impairment of the activation of effector T cells and NK-T cells (58). The activation of antigen specific regulatory T cells leads to an antigen-specific suppressive effect on the anti-tumor immune response and it

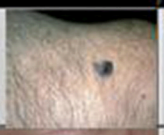
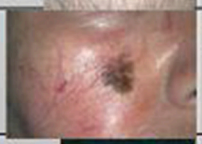
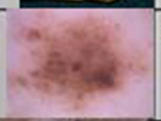
Clinical Type	Chronic sun damage	Sunburns	BRAF	NRAS	KIT
SSM 	no	yes	50%	20%	0%
LMM 	yes	no	10%	10%	2%
ALM 	no	no	15%	15%	15%
MuM 	no	no	5%	15%	20%
UvM 	no	no	0	0 25% GNAQ; 55% GNA11	0

FIGURE 3 | Associations of the clinical variants of CMM with modalities of exposures and driver mutations. Legend: SSM, superficial spreading melanoma; LMM, Lentigo Maligna Melanoma; ALM, acral lentiginous melanoma; MuM, mucosal melanoma; UvM, uveal melanoma (references in the text).

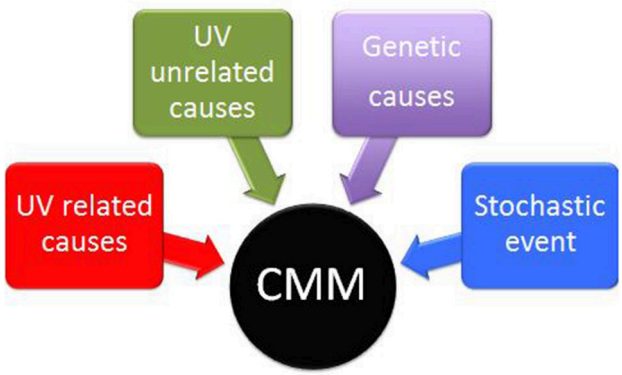


FIGURE 4 | Interplay of UV related, UV unrelated, genetic and stochastic factors in the pathogenesis of cutaneous Malignant Melanoma (CMM).

creates an environment where skin tumors can grow (59, 60). However, some degree of immune-surveillance is still preserved as shown by the evidence that the risk for CMM is much higher for patients who are immune-suppressed by drugs. Therefore, the main difference between UVR-induced immunosuppression and drug-induced immunosuppression is probably to be identified in antigen specificity (54, 60).

A key problem is the identification of the UV dose that can be significantly dangerous in humans (61). In early studies, immunosuppression in mice was reached with chronic exposures at erythemal doses (62). Later on, it was proved that a single high irradiation (above the erythemal dose) was also capable of producing the same effect (61).

Afterwards, it was demonstrated that low UVB doses (lower than the MED) - as well as UVA - could promote immunosuppression both in mice (61) and human beings (23, 63–65). Consequently, it seems that immunosuppression can be obtained even while normally walking outdoors, in daylight, during summer. However, everyone is frequently exposed to a very low dose of UV. Therefore, the most important question becomes: how much these exposures are dangerous for CMM development (61)? Chronic low-dose exposures were not found to represent a risk factor for CMM in melano-competent subjects (2). Even more surprisingly, it was recently found that a history of sunny holidays, before CMM diagnosis, was associated with lower mean Breslow thickness (66) and intermittent or regular sun exposures, after CMM diagnosis, were associated with lower mean relapse rates (66, 67). A possible explanation is that low/physiologic doses of UVR inhibit the adaptive immune system but induce parts of the innate immune system (68). However, UVR effects on the presence and activity of innate immune system cells, e.g., macrophages, tumor associated macrophages (TAMs), dendritic cells (DCs), mast cells and NK cells remain to be explored (69).

UVR, VITAMIN D AND MELANOMA

Vitamin D and its receptor polymorphisms might play an important role as risk factors for CMM (70). It is very well known that UV radiation is essential for Vitamin D synthesis,

in particular for the photoconversion of 7-dehydrocholesterol to cholecalciferol in the epidermis. The production of this vitamin represents one of the most important beneficial effects of sunlight exposure.

Vitamin D bond to its receptor (VDR) results in the transcription of different genes that play a role in the inhibition of MAPK signaling, the induction of apoptosis and cell-cycle inhibition. Therefore, vitamin D has anti-proliferative and pro-apoptotic effects in many kinds of cells, including melanoma cells (71). Vitamin D has also several other positive effects against melanoma, e.g., increase of tumor suppressor PTEN, increase of metastasis suppressor NDRTG1, anti-inflammatory and anti-angiogenic effects and inhibition of "*in vivo*" melanoma cell proliferation, migration and metastasis (71). However, studies about Vitamin D immunological activity apparently show contrasting findings (61). It was found that Vitamin D might have both a suppressive (58) and a protective activity (72). In other studies it was reported that it is not necessary to immune-suppress UV irradiated animals with Vitamin D to induce CMM (58, 73). A possible explanation of these contrasting effects could be found in different vitamin D concentrations and/or particular pre-activated pathways (58). For example: topically applied 0.1 μg of 1,25(OH)2VitD3 (diluted in acetone/olive oil, 4:1), which represents 240 pmoles, seems able to induce immunosuppression. On the other hand, Dixon et al. (72) used 159.6 and 44.8 pmoles (diluted in ethanol, propylene glycol, and water to a final solvent ratio of 2: 1: 1, resp.) of the vitamin, in order to obtain significant protection against UV-induced immunosuppression. Even though the concentration of Vitamin D used in these experiences is different, an important question arises: which one best represents the concentration of vitamin D in the skin after UV exposure? A conclusive answer has not been found so far (73–75) but it seems likely that biological Vitamin D increments after UV exposure are not sufficient to justify the suppression of specific immune responses. Findings of recent clinical studies have suggested (but by no means proved) that vitamin D might also have a role in melanomagenesis and tendency to metastatic dissemination (71). Godar et al. have suggested that low cutaneous vitamin D3 levels with high environmental and low ratio of UVB/UVA doses are the two main drivers for CMM development. In fact, both Europeans and Americans, in some age groups, have a significant increase of CMM incidence if this ratio decreases (76). If this is true, we could explain the curious relationship between melanoma risk and sun exposure, where sunburn is a factor but occupational sun exposure is not (at least in temperate climes). In MM patients, decreased 25(OH)D serum levels are associated with increased tumor thickness and advanced tumor stage (77).

CONCLUSIONS

The life of human beings depends on the sun. This relationship may be beneficial but, at the same time, dangerous. CMM is one of the most deadly tumor and sunlight is, for sure, the main risk factor, although genetic, UV-unrelated and stochastic factors could also play a pathogenetic role (78) (**Figure 4**). Worldwide, CMM incidence is progressively increasing and two over simplified hypotheses are often put forward as possible explanations: (1) increasing sun exposures and (2) increasing aging of the population. However, we have no data to support the first hypothesis (when exposing to the sun, people tend to be more careful now than before), and the increase of life expectancy in western countries in the last 2 decades seems rather stable[1]. In addition, a growing number of data point out that the relationship between sun exposure and CMM is not simple and straightforward. In particular, even if there is not a light dose that can always be considered either dangerous or beneficial, we know that some UVR doses and some UVA/UVB combinations have a better ratio of beneficial rather than dangerous effects. It is reasonable to conclude that the assessment of the optimal UVR exposure level for each individual will be one of the major future challenges.

AUTHOR CONTRIBUTIONS

PC-P, MV, MA, AZ, SC, and CZ researched literature to gather necessary information and contributed in the writing of the paper. PC-P, MV, MA, AZ, SC, CR, and CZ reviewed the article several times to reorganize the concepts and provide a more solid structure and reviewed the bibliography.

[1]http://www.worldlifeexpectancy.com/world-life-expectancy-map.

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MINI REVIEW
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Polymorphic Light Eruption: What's New in Pathogenesis and Management

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Polymorphic light eruption is the commonest photosensitive disorder, characterized by an intermittent eruption of non-scarring erythematous papules, vesicles or plaques that develop within hours of ultraviolet radiation exposure of patient skin. Together with the lesions, a terrible itch starts and increases with the spreading of the disease, sometimes aggravated by a sort of burning sensation. Clinical picture and symptoms can improve during the rest of the summer with further solar exposures. In the last years many advances have been performed in the knowledge of its pathogenesis and some news have been proposed as preventive, as well as therapeutic options. All this has been discussed in the current mini review.

Keywords: polymorphic light eruption, photosensitive disorders, phototherapy, apoptosis, delayed type hypersensitivity reaction

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INTRODUCTION

During winter, sometimes dermatologists receive asymptomatic patients, with no specific lesions other than, perhaps, some post-inflammatory discoloration, but with a desperate need for help. They start to tell the story of a number of papules or vesicles appearing on their skin after the first intense sun exposure of the year. Together with the lesions, a terrible itch starts and increases with the spreading of the disease, following the next sun exposure, sometimes aggravated by a sort of burning sensation. Nevertheless, this people are model sun seekers and continue to enjoy the sunshine throughout the summer, waiting for the papules to gradually fade and disappear. The main questions are: how can I prevent this? Why I'm getting this problem since "5" years now, but I never had it before?

We are most probably dealing with polymorphic light eruption (PLE) and, following the requests of our patients, medical research has mainly been focused on prevention strategies become nowadays quite satisfactory. On the other side, the second and certainly less explored question remains unclear, unless multiple pieces have been added to this rather complicate puzzle. Aim of this brief review is to resume the most recent advances in PLE possible mechanisms and the most used protocols for prevention or treatment.

PATHOPHISIOLOGY OF POLYMORPHIC LIGHT ERUPTION: WHAT'S NEW?

PLE is the commonest photosensitive disorder, characterized by an intermittent eruption of non-scarring pruritic erythematous papules, vesicles or plaques (**Figure 1**) that develop within hours of ultraviolet radiation (UVR) exposure of patient skin. The disease is dependent on genetic susceptibility, as well as environmental component, such as type of exposure. PLE appears to cluster in families: it has been estimated that the prevalence of PLE was 21 and 18% in monozygotic and dizygotic twins, respectively (1). Moreover, a positive family history of PLE in first-degree relatives was present in 12% of affected twin pairs respect to 4% of unaffected twin pairs ($p < 0.0001$). The probandwise concordance in monozygotic was superior than in dizygotic twin pairs (0.72 vs. 0.30, respectively), demonstrating a strong genetic effect (1). Many genes of potential interest in the pathogenesis of PLE have been investigated with generally unrewarding results. Using segregation analysis, it has been estimated that 72% of the UK population carry a low penetrance PLE susceptibility allele (2).

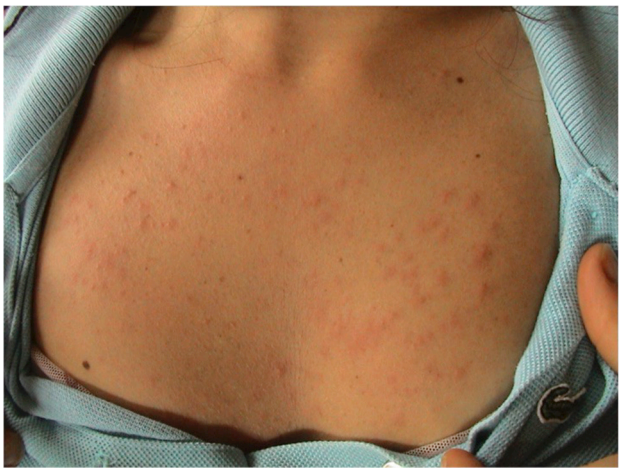

FIGURE 1 | Clinical picture of polymorphic light eruption in a young woman.

The Failure of Apoptosis: the Possible Photo-Induced Neo Antigens

In a recent genome-wide expression analysis, only 16 genes were differentially expressed between PLE and healthy controls after UV irradiation respect to control (3). Of these genes, 14 showed lower expression in PLE patients, whereas two resulted over-expressed. Among the 14 genes with lower expression in PLE are: complement 1s subunit (C1s), scavenger receptor B1 (SCARB1) fibronectin (FN1), immunoglobulin superfamily member 3 (IGSF3), caspase-1 (CASP1) and paraoxonase 2 (PON2), all genes associated with apoptotic cell clearance. It has been supposed that protein modification during apoptotic cell clearance could lead to potential auto-antigen formation (4). Then, the reduced expression in PLE patients of genes connected to this process might represent a possible auto-antigen source, as well as a crucial phase in the initiation of the autoimmune process that promotes the disease (3). In accordance to these findings, Kuhn et al. showed accumulation of apoptotic cells in PLE patients irradiated either with 1.5 Minimal Erythema Dose (MED) of UVB, or 60–100 J/cm^2 of UVA1, compared to controls (5).

Immunity: Tolerance's Failure

Auto-antigens deriving from the inefficient clearance of apoptotic cells, are probably taken up by dendritic cells (DCs) and presented to naive T-cells (cytotoxic and helper) thereafter transformed in auto-reactive T cells (6, 7). This partial failure of the apoptosis contributes, together with the inadequate immunosuppression after UV exposure, to the antigen recognition and presentation, leading to the clinical manifestation typical of PLE patients (8). Indeed, the failure of normal UVR-induced immunosuppression has been proved as the main immunological abnormality in PLE, explained, initially, by the permanence of Langerhans cells (LCs) in the epidermis. This over-activation of the immune system, which escapes to the functional UV-induced tolerance, is probably responsible for the reduced skin cancer prevalence in PLE patients (9). On the other hand, the same mechanism is guilty for the failure of allergic contact dermatitis (ACD) suppression, after UVR exposure (10).

Inflammatory Pathway: Delayed-Type Hypersensitivity Reaction

The immunological mechanisms involved in PLE, with mediators from the innate and adaptive immune system, are very similar, either from the histological or the biochemical point of view, to the ACD ones. In effect, in the early seventies, Epstein first indicated PLE as a delayed-type hypersensitivity reaction (DTHR) to undefined UVR-induced cutaneous antigen (11). Recently, to reinforce this concept, some of the inflammatory mediators involved in ACD have been demonstrated also in PLE. For example, IL-1 family (12, 13), a growing group of cytokines that play several roles in immune regulation and inflammation (14), involved also in ACD pathogenesis (12, 15), has been explored also in PLE (16). IL-36α and IL-36γ, the pro-inflammatory members of IL-1 family were increased in PLE respect to controls, as for ACD samples, but IL-36γ was much enhanced in PLE than in ACD (16). Acting through the common receptor composed of IL-36R and IL-1R/AcP (IL-1RL2), IL-36α, IL-36β, and IL-36γ activate NF-κB and MAPKs, promoting inflammatory reactions. The increase of IL-36s in skin and peripheral blood of PLE patients indicates the activation of local and systemic immune response, as found in multiple inflammatory skin conditions (15, 17, 18). Probably,

Abbreviations: IL, Interleukin; PLE, Polymorphic light eruption; ACD, Allergic contact dermatitis; DTHR, Delayed type hypersensitivity reaction; UVR, Ultraviolet radiation; C1s, Complement 1s subunit; SCARB1, Scavenger receptor B1; FN1, Fibronectin; IGSF3, Immunoglobulin superfamily member 3; CASP1, Caspase-1; PON2, Paraoxonase 2; MED, Minimal Erythema Dose; DCs, Dendritic cells; LCs, Langerhans cells; TLR, Toll like receptor; AMPs, Antimicrobial peptides; SPF, Sun protection factor; PUVA, Psoralen and UVA therapy; NB-UVB, Narrowband; BB-UVB, Broadband UVB; MPD, Minimum phototoxic dose; Tregs, Regulatory T cells; PL, Polypodiumleucotomos.

the link between IL-36s and UVR exposure is represented by the paracrine pro-inflammatory signal of toll like receptor (TLR)-3 activation, due to the release of RNA by necrotic keratinocytes (19). Indeed, the failure of apoptotic clearance in PLE, with abundance of cellular debris, could be responsible for an amplification of this "alert signal."

Moreover, IL-36s could contribute to amplify the innate immune signal and the consequent inflammatory cascade, promoting antimicrobial peptides (AMPs) (20).

Inflammatory Pathway: AMPs and Microbiome

As largely examined in multiple skin inflammatory processes, these mediators, named as defensins (α and β), cathelicidin (LL37), ribonuclease 7 (RNase7) and psoriasin (S100A7), in light of the imbalance induced by UVR on keratinocytes and skin microbiome, have also been investigated in PLE (21, 22). Patra et al. have found that the expression of psoriasin, RNase7, HBD-2, and LL-37 was increased in PLE lesional skin, whereas HBD-3 was decreased. Considering the skin surface as a "multiethnic world," without forgetting the crucial role of keratinocytes, we can't exclude that AMPs release could be determined by modification in microbiome components after UV interaction (23). Indeed, microbiome could represent the source, direct or indirect, of the yet undetected UVR-induced antigens formed in PLE patients, leading to keratinocyte damage. As a consequence, LL-37, also induced by UVB, IFNγ, TNF-α, IL-6, could represent a potential indirect driver of PLE (23). It can form aggregates with self-nucleic acids able to activate pDCs: in psoriasis it has been recognized as the main autoantigen (24). Even though in PLE patients a complete absence of pDCs has been reported (25), an autoimmune milieu exists, and LL-37 could play a pivotal role, inducing other inflammatory pathways. In **Figure S1** (Supplementary Material), the concepts expressed above are visualized in a cartoon.

Therapy of Polymorphic Light Eruption: What's New?

The first line of treatment for PLE includes sun avoidance, sunscreens and topical corticosteroids (26). For all patients preventive management is fundamental during sunny weather, by avoidance of intense UVR exposure and use of protective clothing, as well as application of sunscreen, in particular during the first exposure of the year. New generation broad-spectrum sunscreens, with high sun protection factor for UVB (SPF), together with longer wavelength UVA protection, have been reported to confer total or partial protection in up to 90% of PLE patients (27, 28). The use of oral antioxidants and nicotinamide could represent an additional valid preventive measure for these patients. The beneficial effects of nicotinamide have been investigated in an uncontrolled trial of 42 patients, where 60% of them reported complete abolition of symptoms when taking 2–3 g of nicotinamide daily, before sun exposure (29). Moreover, an extract of the tropical fern Polypodiumleucotomos [PL) has been shown to exert both potent antioxidant and immunomodulatory effects. When administrated at 480 mg/daily before sun exposure it significantly reduced skin reactions and subjective symptoms (30, 31). Regarding topical corticosteroid, even if no trials have been made to determine their efficacy in PLE, they are widely used to reduce itch (26). The second line of treatments for PLE includes systemic corticosteroids and photo(chemo)therapy (26). In a randomized, double-blind, placebo-controlled trial (32) the authors suggested the use of 25 mg prednisolone daily for 4–5 days at the onset of the eruption. Although, the potential long-term side effects of repeated courses of prednisolone must be considered, it could be advised for patients who suffer from occasional attacks of PLE, in the absence of any contraindications. In milder cases of PLE, a self-conditioning programme by graduate exposure to sunlight in springtime may be sufficient (33). Whereas, in more severe cases, medically supervised conditioning/desensitization treatment may be more appropriate. A course of psoralen and UVA therapy (PUVA), narrowband (NB)-UVB or broadband (BB)-UVB phototherapy, usually administered in early spring, can be effective as well as prophylactic treatment (26). Treatment protocol generally consists of one course of phototherapy/photochemotherapy over 5–6 weeks. Starting doses depend on minimal erythemal dose (MED) or minimum phototoxic dose (MPD), and are frequently 50–70% of these measured thresholds with incremental increases. To maintain the benefit acquired with the desensitizing therapy, a regular sun exposure throughout summer is advised, otherwise the hardening could be lost within 4–6 weeks. In the treatment of PLE, NB-UVB should be preferred to PUVA (strength of recommendation D; level of evidence 4), because of the lower risk of photocarcinogenesis, no risk of nausea or other side-effects associated with the ingestion of MOP, and no need to use post-treatment eye protection. However, PUVA should be considered, before other systemic treatments, if NB-UVB has failed or has previously triggered the eruption. In effect, as described below, the efficacy has been proved for multiple phototherapy regimens (BB-UVB, NB-UVB and PUVA), and side-effects, in term of rash provocation, erythema and itch were found to be more common with UVB than with PUVA (34). As summarized, in the literature, the efficacy of PUVA results in a 65–100% photoprotection rate (34). Multiple comparative studies have been performed, but from the only randomized controlled trial between PUVA and NB-UVB plus placebo tablets, three times a week, for 5 weeks, no significant difference in efficacy emerged, considering occurrence of PLE or outdoor activity restriction (35). In the 10 years retrospective review, reported by Man et al. (36), 170 patients with moderate-to-severe PLE received PUVA and/or UVB phototherapy. In detail, 8 patients received PUVA, 128 NB-UVB, 5 BB-UVB, and 29 patients, who failed to respond satisfactorily to NB-UVB, were given PUVA the following year. Self-assessments were made of the severity, and frequency of PLE episodes were reported at the follow up visits in autumn or during the following spring. Good or moderate improvement was reported in 88% of patients treated with PUVA and in 89% who received UVB. Of the patients treated with both PUVA and NB-UVB, the majority preferred PUVA. In another 14-years retrospective study on 79 patients treated with phototherapy (37), the efficacy, measured during the following summer in term of

photoprotection with complete/partial remission, was 65% for PUVA, 82% for BB-UVB and 83% for UVA alone. In this case the treatment with PUVA was reserved to more severe PLE forms.

The mechanisms by which phototherapy induces photoprotection are not fully understood.

However, in the last years many advances have been performed. In addition to the well-known effects on melaninization and epidermal thickening of phototherapy, a wide range of UV induced immunomodulatory and anti-inflammatory properties are reported (38). Both UVB and UVA modulate adhesion molecule expression and induce soluble mediators, such as a-melanocyte-stimulating hormone, IL-10 (which suppresses the production of interferon γ) and prostaglandin E2, that explicate anti-inflammatory actions, preventing T cells activation and promoting apoptosis of skin infiltrating T cells (34). Moreover, it has been demonstrated that prophylactic UV photohardening in PLE patients restores the UV-induced LC migration from the epidermis to the skin-draining lymph nodes: one of the key cellular event in UV-immunosuppression (39). The tolerance induced by LC is mediated by the release of immunosuppressive cytokine such as IL-10, and by the interference with maturation and induction of regulatory T cells (Tregs) (40). Moreover, recently, an interesting link has been reported among LC, Tregs and vitamin D3. Indeed, it has been demonstrated that a short-term 1 week topical pre-treatment with the 1,25-dihydroxyvitamin D analogue, calcipotriol, diminished PLE symptoms after subsequent experimental photoprovocation (41). In addition, in a murine study 1,25-dihydroxyvitamin D showed comparable immunosuppressive effects as UV (42). Another interesting crosstalk has been highlighted between LCs and mast cells. In addition to their recognized role in atopy, dermal mast cells are also responsible for protecting the skin from UVB-induced inflammation, promoting UV immunosuppression (40). Human studies have demonstrated that after acute and chronic UVR exposure, dermal mast cells number increases, together with the release of IL-10. Overall these data suggest a potential role for mast cells in PLE, and in the mechanism of photohardening. In accordance with this, Wolf et al. have reported, for the first time, that photohardening significantly increases mast cell density in the papillary dermis of PLE patients (40). Summarizing, photohardening works in PLE by restoring the normal UV immune suppressive pathway, involving multiple cell types. The third line treatment for PLE includes the use of systemic immunosuppressive drugs, such as azathioprine and cyclosporine. However, only sporadic cases of patients successfully treated are reported in literature (43, 44). Moreover, hydroxychloroquine, omega-3 fatty acids, and beta-carotene have been proposed as treatments, but further double-blind, randomized controlled trials to really assess their clinical efficacy are required.

CONCLUSIONS

Since the high prevalence and increasing incidence of PLE, associated to discomfort and life style restrictions, future studies are necessary to find novel therapeutic and/or preventive strategies. The choice of the appropriate PLE treatment requires a good knowledge of the individual clinical course of the disease together with the possibility of performing phototest. Some new aspects in the possible activation and promotion of the inflammatory process have been highlighted.

To the current state of knowledge, despite the identification of some crucial cellular regulation involved on the restoration of the immune tolerance, it is difficult to draw definite conclusions about the efficacy of various potential treatments in PLE, due to lack of adequate studies and the difficulty in assessing outcome measures. The clinical score to assess PLE severity (PLESI) (45) remains an instrument scarcely used and mainly restricted to research purposes. The deeper study of the underlying pathogenetic mechanisms of the disorder will permit a more targeted treatment approach.

AUTHOR CONTRIBUTIONS

SL projected the manuscript, selected the material for the paper, wrote the initial draft and corrected the following drafts of the manuscript. AR was engaged in the writing of the manuscript, supporting new ideas of contents and style.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2018.00252/full#supplementary-material

Figure S1 | Interplay between innate and adaptive immune system in a context of apoptosis failure in the epidermis. (Green symbol) Psoriasin: abundant expression in spinous and granular layers of PLE skin. (Blue symbol) RNase7: mainly expressed in keratinocytes of the stratum granulosum and stratum corneum of PLE lesions. (Yellow symbol) LL-37 was profoundly expressed in and around blood vessels and glands in PLE. (Violet cell symbol) Apoptotic keratinocytes with inefficient clearance.

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Novel Means for Photoprotection

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Due to changes in human lifestyle (expanded sunbathing, the use of solaria, etc.) and, most importantly, increasing lifetime and thus higher cumulative exposure to solar radiation, skin aging and skin cancer have become major health issues. As a consequence effective photoprotection is of outmost importance to humans. In this regard a lot has been learned in the past about the cellular and molecular basis underlying ultraviolet (UV) radiation-induced skin damage and, based on this knowledge, numerous skin protective approaches including organic and inorganic UV-filters, but also topically applicable antioxidants, DNA repair enzymes and compatible solutes as well as oral photoprotective strategies based on nutritional supplements have been developed. A new aspect is here that sun protection of human skin might even be possible after solar radiation-induced skin damage has occurred. A second, very important development was prompted by the discovery that also wavelengths beyond the UV spectrum can damage human skin. These include the blue light region of visible light (VIS) as well as the near infrared range (IRA) and corresponding sunprotection strategies have thus recently been or are still being developed. In this article we will provide a state of the art summary of these two novel developments and, at the end, we will also critically discuss strengths and weaknesses of the current attempts, which mainly focus on the prevention of skin damage by selected wavelengths but greatly ignore the possibility that wavelengths might interfere with each other. Such combined effects, however, need to be taken into account if photoprotection of human skin is intended to be global in nature.

Keywords: photoprotection, visible light, blue light, red light, infrared, Ultraviolet-B, Ultraviolet-A, DNA repair enzyme

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INTRODUCTION

Reflected and filtered by the atmosphere only a part of sun light reaches the surface of the earth. This radiation can induce harmful effects on human skin including sunburn, immunosuppression, photoaging and skin cancer. It is generally thought that high-energetic Ultraviolet radiation (UVB, 280–315 nm and UVA, 316–400 nm) is mainly responsible for these adverse effects. As a consequence, traditional photoprotection of human skin was restricted to protection against UV-rays. More recently, this view has been changed by an increasing number of independent scientific reports indicating that (i) also wavelengths of the solar spectrum beyond UV radiation, VIS (400–770 nm) and near infrared radiation (IRA, 771–1,440 nm), can damage human skin and (ii) that there is growing evidence that photoprotection is also possible after sun-induced skin damage has occurred. Here, we will summarize these novel developments and will critically discuss strengths and weaknesses of existing approaches. We will conclude by providing our view on upcoming challenges, which we believe, will further improve the performance and efficacy of sun protection of human skin.

PROTECTION AGAINST IRA

IRA is the major component of natural sunlight and approximately 30% of the total solar energy reaching the earth's surface is within the IRA range. Traditionally, photodermatology focused mainly on physiological, pathophysiological and therapeutical effects of UVB- and UVA-radiation whereas wavelengths in the IRA range have long been ignored. In recent years, however, the number of studies addressing IRA-induced skin damage increased and today, it is generally accepted that wavelengths in this range similar to UV radiation (UVR) can induce skin damage. Skin damage caused by IRA radiation mainly manifests as perturbation of extracellular matrix homoeostasis by degrading dermal connective tissue which clinically presents as wrinkle formation. These findings have been recently reviewed in great detail and we will therefore only mention important key studies for the purpose of this review.

The impact of IRA on human skin is best illustrated by Calles et al. who showed that approximately 600 genes are IRA responsive in human dermal fibroblasts. By functional clustering these identified genes could be assigned to groups involved in extracellular matrix homeostasis, apoptosis, cell growth and cellular stress response (1). In line, additional studies addressing IRA-induced skin damage reported of an increased expression of matrix degrading enzymes such as MMP-1 and MMP-9 (matrix metalloproteinase-1/9), along with a decreased collagen production (2–4). Some of these studies were criticized by using irradiation doses, which exceed physiological doses a human being is usually exposed to natural sunlight. Of note, however, similar effects have been reported using low or moderate doses of IRA *in vitro* (5). More importantly, these findings were underlined by a recent study of Cho et al. in which natural sunlight was filtered to allow for study of IRA and heat (4). In aggregate, all these studies show that IRA-irradiation can cause wrinkle formation by enhancing the expression of matrix degrading enzymes.

An obvious approach for an effective protection against IRA-induced skin damage would be the use of physical or chemical filters similar to classical UV protective sunscreens. Regularly used compounds, however, have not been shown to possess significant IRA-filtering capacities (6). Although, inorganic pigments with IR-reflecting properties are well known, e.g., coloring pigments used as roof coatings, these substances have a major disadvantage because they would be visible to the consumer after topical application (7). Alternatives that might cause less or no compliance problems could be formulations containing fumed silica which disperse and block infrared radiation (8). Of note, many studies provide evidence that IRA-induced skin damage is mediated around the generation of ROS (reactive oxygen species) (2, 9, 10). Therefore, photoprotection of human skin against wavelengths in this range now mainly involves topically applied antioxidants. In this regard, it is important to emphasize that IRA photoprotection requires specific antioxidants as it could be shown in a target-driven *in vitro* screen in primary human skin fibroblasts. By using IRA-induced MMP-1 mRNA expression as a read-out model certain polyphenols and vitamins could be identified as effective compounds. This was confirmed *in vivo* by topical application of an antioxidant mixture 20 min before IRA-exposure (2). This study actually prompted the development of topical sunscreen products with an efficient protection against IRA, which were first launched in Germany. In the meantime, additional studies have been performed pointing out the necessity of sunscreens, which offer an efficient protection against IRA (3, 10–12). Today, IRA photoprotection is no longer limited to sunscreens but similar to UV protection it may be found in daily skin care products as well (13). Although antioxidants are less potent in preventing sunburn in contrast to classical sunscreens (14) appropriate concentrations of orally administered antioxidants might represent an alternative. These compounds have the advantage that in contrast to topical antioxidants, which might poorly penetrate into the skin and be unstable, the entire skin surface is protected without being affected by washing, perspiration or rubbing.

A persistent major challenge for the development of antioxidants for IRA protection of human skin results from the fact that no standardized *in vitro* or *in vivo* test exists to validate photoprotective properties. Whereas, erythema and pigmentation represent easy to measure biological endpoints for UVA or UVB sunscreens, no endpoints have been identified for IRA, which can be measured non-invasively in human skin. Therefore, we recommend to use IRA-induced MMP-1 mRNA expression in human dermal fibroblasts to screen selected antioxidants in a first step. In a second step, a clinical study should be performed in which complete sunscreen products containing candidate molecules which are proven to be effective *in vitro* are tested as formulations to assess their potential to inhibit IRA-induced MMP-1 mRNA expression in human *ex vivo* skin models or, ideally, *in vivo* in human skin.

PROTECTION AGAINST VIS

Visible light is defined as part of the electromagnetic spectrum that ranges from violet (400 nm) to profound red (770 nm). In contrast to numerous studies which addressed IRA-induced skin damage the number of studies centered around VIS and skin is limited to a few.

Zastrow et al. reported of an increased radical formation analyzed by electron spin resonance in *ex vivo* human skin after irradiation not only with UVR and IRA but also in the VIS range (15). This could be confirmed and extended by a second study using Electron paramagnetic resonance spectrophotometry *ex vivo* and *in vivo* (16). In human epidermis models, VIS is able to induce MMP-1 as well as TNF-α (tumor necrosis factor alpha) mRNA expression in keratinocytes by an increased production of ROS. This increased radical formation was confirmed in *in vivo* human skin by Raman spectroscopy (17). Direct biological consequences of VIS-irradiation on human skin were first shown by Pathak et al. These authors provided evidence that wavelengths in the VIS/long-wavelength UV range

at physiological relevant doses can cause pigmentation *in vivo* in human skin (18, 19). This could be confirmed by a recent study using an artificial irradiation device without contaminating UVR rays and an emission spectrum mainly containing wavelengths between 400 and 800 nm. Of note, increased pigmentation occurred only in darker pigmented skin types ≥III according to Fitzpatrick scale (20). Similar results were observed and could be extended in an independent study in which a marked and prolonged skin pigmentation was induced by blue-violet light in a dose dependent manner, whereas red light did not induce any pigmentation. Compared to UVB-induced hyperpigmentation, blue-violet light induced a more pronounced pigmentation that lasted up to 3 months and histological stainings revealed decreased levels of p53 and necrosis of keratinocytes (21). The absence of p53 activation in pigmentation after blue light irradiation suggests mechanisms, which are different from those known to be involved in the response to UVB. Accordingly, a recent study showed that melanocytes are directly affected by blue light and increase melanin synthesis in response to blue light-induced activation of Opsin-3 receptors on their surface. This mechanism is calcium dependent and involves a kinase-dependent signaling cascade leading to the activation of the transcription factor MITF (Microphthalmia-associated transcription factor) and further to an increased expression of melanogenesis related tyrosinase and dopachrome tautomerase. These enzymes form a complex which is mainly induced in dark skin melanocytes and leads to sustained tyrosinase activity (22). There is also indirect evidence that exposure to VIS can worsen melasma. An iron oxide containing sunscreen providing protection against UVB/UVA plus VIS proved to be superior to a control sunscreen with identical UVB/UVA but without VIS protection in the prevention of melasma relapse (23). There is currently no evidence that VIS can cause health effects beyond skin hyperpigmentation/melasma. In particular, VIS has not been shown to cause wrinkle formation.

Of note, ROS formation and accumulation is a key mechanism for the expression of keratinocyte-derived cytokines, but VIS-induced pigmentation does not involve ROS formation and cannot be targeted by antioxidants. Therefore, to the best of our knowledge, protection against VIS in terms of pigmentation may only be provided by scattering or reflecting VIS in the blue-violet range. The absorption or reflection range of commonly used inorganic sunscreen agents like iron oxide, titanium dioxide or zinc oxide, ranges from UVR to VIS but greatly depends on the particle size. Only optically opaque sunscreen formulations containing inorganic pigments are able to reflect and scatter VIS (24) but these compounds are water-insoluble and leave a white or tinted coating on skin which is unacceptable for most costumers. This was confirmed by a study, which assessed the protective efficacy of several sunscreens containing titanium dioxide and iron oxide against VIS-induced pigmentation in darker pigmented skin types. Here, pretreatment of skin with a VIS-filtering sunscreen based on inorganic compounds reduced VIS-induced pigmentation up to 5 days after exposure (25).

However, in order to develop highly efficient sunscreens against VIS which are also consumer compatible further basic research is clearly needed.

PHOTOPROTECTION AFTER SUN EXPOSURE

A completely new approach is the concept that photoprotection is also possible even after skin damage has occurred. The main goal of such protection strategies is to support or enhance DNA repair by supplying biological active enzymes imbedded in an absorbable formulation.

This can be achieved by the presence of DNA repair enzymes in after-sun lotions or creams, which has been shown to work in a study of Stege at al.. Topical treatment of human skin with liposomes containing active photolyase and subsequent exposure to photoreactivating radiation led to an enhanced removal of UVB-induced cyclobutane pyrimidine dimers, diminished erythema and sunburn-cell formation as well as suppresses UV-induced expression of ICAM-1 (intercellular adhesion molecule-1), an enzyme which is required for inflammatory immune response in the epidermis (26). In another clinical study, Wolf and co-workers have demonstrated that liposomes containing the DNA repair enzyme T4 endonuclease prevent the UV-induced upregulation of immunosuppressive cytokines in patients with a history of skin cancer. The repair enzyme penetrated into the human skin and was located in keratinocytes and epidermal Langerhans' cells (27). Similar formulations were also tested in several clinical studies on the prevention of actinic keratosis (AK). By treatment of the precancerous field of AK with a medical device containing conventional UV-filters and biological active photolyase a significant general improvement of the skin was observed and an over-expression of fundamental processes related to tissue reconstruction, e.g., cell communication, signaling and adhesion could be demonstrated (28). In line with these observations, a 9-months randomized clinical study analyzed the impact of a sunscreen containing photolyase on patients with AK after photodynamic therapy. Compared with a conventional sunscreen the daily application of sunscreen plus photolyase was associated with a significant prevention of new AK lesions. During treatment, no additional phototherapy was required in the photolyase group, whereas newly AK lesions developed in the group receiving sunscreen only (29). This strongly indicates that DNA repair enzymes used in sunscreens are able to prevent the development of AK's in human skin.

OUTLOOK

Traditionally, the majority of photodermatologic studies analyzed each wavelength range, i.e., UVB, UVA, VIS or IRA-induced biological effects on human skin, separately. However, human skin is naturally exposed to all of these wavelengths simultaneously, and it is conceivable to assume that interactions or interferences between these wavelengths exist that may fundamentally influence the overall biological response and therefore are of utmost importance for the development and improvement of photoprotection.

Support of this concept was first provided by Schieke et al. who investigated the molecular crosstalk of UVA and UVB on activation of MAPK (mitogen-activated protein kinase).

In a first step, the activation pattern after single irradiation with UVA or UVB was analyzed resulting in a UVA-induced modest and transient phosphorylation of ERK 1/2 (extracellular signal-regulated kinase-1/2), 15–30 min after exposure whereas UVB irradiation caused a strong and immediate activation that lasted up to 1 h. Activation of p38 and JNK 1/2 (c-jun N-terminal kinases-1/2) was only slightly enhanced after single irradiation. A different pattern was observed if keratinocytes were sequentially exposed to UVA and UVB. In this case, p38 and JNK 1/2 phosphorylation were enhanced, but the UVB-induced immediate activation of ERK 1/2 was prevented, regardless of the irradiation sequence (30). This study has shown that a molecular crosstalk of UVB and UVA exist which has been observed on level on MAPK signaling and may represent and evolutionary conserved defense strategy of human skin cells to respond to solar radiation-induced stress. A second study was published in 2007 demonstrating that apoptosis after simultaneous irradiation with UVB+UVA (solar simulated UVR) in comparison to single UVB is ameliorated in a UVA dose dependent manner *in vivo*. Here, histological analysis of sunburn cell formation and caspase-3 activation revealed that apoptosis in mice can be reduced up to 50% after 24 h post-exposure to 3MEdD (minimal edematous dose) of solar simulated UVR. This effect is probably mediated by increased heme oxygenase activity, an enzyme which plays an important role in the protection against oxidative stress in human skin (31). Of note, different ratios of UVA/UVB, which were used for irradiation in this study, are of high physiological relevance concerning the altering emission spectrum of the sun, which is strongly affected by daytime, weather conditions and the season. In addition to apoptosis, also UVB-induced immunosuppression was shown to be ameliorated after irradiation with solar simulated UVR *in vivo*. Interleukin-6 which is released after UVB could be identified as an essential factor of the UVA-mediated protective effect on the immune response (32). There is also evidence that crosstalk signaling may exist for UVB and IRA radiation, although in most of these cases the sequence of irradiation (first UVB subsequently IRA vs. first IRA subsequently UVB) fundamentally influenced the biological response (reviewed in Grether-Beck et al.). In aggregate, these results strongly indicate that simultaneous UVB+UVA irradiation causes a third biological response, which differs from single UVB or UVA exposure, and even more important cannot be explained by a simple addition of biological effects.

These examples emphasize the need for detailed simultaneous irradiation studies targeting the analysis of the relative contribution of each wavelength to the entire biological effect of solar radiation-induced skin damage. Considering that human skin has perfectly adapted to natural sunlight during evolution then the exposure to the complete solar spectrum provides an optimized stress response with the overarching goal to limit skin damage as much as possible. Therefore, studies which use irradiation protocols to single wavelengths only or merely sequentially add two or more wavelengths ranges may lead to results which are of limited physiological relevance or are completely misleading. We therefore believe that this issue can be best assessed by the development of a novel irradiation device which (i) allows simultaneous irradiation with UVB, UVA, VIS and IRA at physiologically relevant doses *in vitro* and *in vivo,* (ii) but to selectively dim off specific wavelengths areas to understand their relative contribution to the entire biological effect and (iii) to change continuously the intensity of each installed lamp especially in the range of UVB and UVA to simulate variations of the emission spectrum as it is the case for natural sunlight during daytime or seasons. As a consequence, we have recently built this irradiation device, which is currently used in *in vitro*, *ex vivo* and *in vivo* studies to better understand the interaction of different wavelengths present in natural sunlight.

AUTHOR CONTRIBUTIONS

KS: literature research, writing and drawing of figures; JK: literature research and writing. All authors read and approved the final version of the manuscript.

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Oral Photoprotection: Effective Agents and Potential Candidates

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Electromagnetic radiation in the ultraviolet, visible, and infrared ranges produces biologic effects in humans. Where some of these effects are beneficial, others are harmful to the skin, particularly those stemming from ultraviolet radiation (UVR). Pharmacological photoprotection can be topical or systemic. Systemic photoprotection is often administered orally, complementing topical protection. New and classic oral agents (e.g., essential micronutrients as vitamins, minerals, polyphenols, carotenoids) are endowed with photoprotective and anti-photocarcinogenic properties. These substances bear the potential to increase systemic protection against the effects of electromagnetic radiation in the UV, visible, and infrared ranges. Protective mechanisms vary and include anti-oxidant, anti-inflammatory, and immunomodulatory effects. As such, they provide protection against UVR and prevent photo-induced carcinogenesis and aging. In this review, we present state of the art approaches regarding the photoprotective effects of vitamins and vitamin derivatives, dietary botanical, and non-botanical agents. A growing body of data supports the beneficial effects of oral photoprotection on the health of the skin. More studies will likely confirm and expand the positive impact of oral dietary botanicals as complementary measures for photoprotection.

Keywords: oral photoprotection, oxidative stress, dietary botanical, photodamage, photocarcinogenesis

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INTRODUCTION: PHOTOPROTECTIVE AGENTS

Sunscreen-based photoprotection is a major part of the first line of prevention to combat photoaging and skin cancer. Topical photoprotection is usually carried out by applying a thin ultraviolet radiation (UVR)-absorbing layer on the skin before sun exposure. Despite the incorporation of new technology and innovative approaches in topical photoprotection, inadequate use, and lack of optimization still limit usefulness of sunscreens. Topical sunscreens also have intrinsic limitations, among them, chiefly short half-life on the skin, which highlights the need for frequent reapplication, a lack of systemic efficacy, and potential side effects (1, 2). Despite the widespread use of sunscreens, sunburn remains commonplace.

Conversely, oral photoprotectors do not directly protect the skin against the damage induced by high energy photons; therefore, they are not very effective against the erythema and other deleterious effects caused by the sun. However, they do possess several advantages, mainly their ease of use. Also, their efficiency is not altered by external conditions, their half-lives can be determined pharmacologically, and their effects do not depend on the degree of absorption through the skin.

The ideal photoprotective agent would be an oral photoprotector with cutaneous affinity. The overarching idea is that oral photoprotective agents need to provide uniform protection of the skin to be useful in the primary prevention of skin cancer and photoaging (1).

These oral photoprotective products usually contain one or more active principles that activate different mechanisms of photoprotection, especially those related to their anti-oxidant actions (1, 3). These substances act by increasing the anti-oxidant efficacy of the body following the loss of endogenous anti-oxidants after UVR exposure. UV radiation induces DNA damage, triggers inflammatory phenomena, and promotes tumor growth. It also contributes to aging through alterations in collagen remodeling and mitochondrial deletion. Most of these detrimental effects are mainly mediated by oxidative stress (2). Some of these substances also reduce UVR-induced immunosuppression.

The following sections provide an update of state of the art regarding the properties of oral photo protectors.

VITAMIN DERIVATIVES WITH ANTI-OXIDANT PROPERTIES

Single Vitamins (Table 1)

Carotenoids

Carotenoids are pigments existing in a wide variety of vegetables and fruits, especially in tomatoes. They also appear in considerable amounts in human plasma and tissues. However, carotenoids are exclusively synthesized by plants. Hence those appearing in animals and humans have been acquired through the diet. Carotenoids decrease reactive oxygen species (ROS) in aerobic metabolism (29).

About 50 variants of carotenes are present in a typical human diet and, of these, six are found mainly in the blood: α-carotene, β-carotene, zeaxanthin, lutein, β-cryptoxanthin, and lycopene. Of these, lycopene is the most efficient regarding anti-oxidant activity (30). *In vitro* and *in vivo* studies have revealed that carotenoids can suppress UVA and UVB-mediated ROS formation, thereby, preventing photoinactivation of anti-oxidant enzymes, lipid peroxidation, and induction of DNA damage caused by oxidative stress (30, 31).

Lycopene

Lycopene is the predominant carotenoid present in tomatoes and other vegetables and red fruits, except in cherries and strawberries. Lycopene, a polyunsaturated hydrocarbon (C40H56), is endowed with a very high anti-oxidant capacity quenching singlet oxygen (32). *in vitro* studies with human skin fibroblasts disclosed a reduction of UVB-induced lipid peroxidation by lycopene (33).

Several investigators have reported on the effects of lycopene in humans. Subjects treated with oral lycopene for 10 weeks had 40% less dorsal erythema formation in response to UVR compared to untreated subjects (6), as measured by chromametry (6). Similarly, an intervention study in which healthy women received tomato paste rich in lycopene during 12 weeks supplemented with olive oil suggested that lycopene exerted beneficial properties (7). Lycopene reduced matrix metalloproteinases 1 (MMP-1) overexpression and mtDNA 3,895-bp deletion produced by UVR. The mechanism proposed for lycopene relates to its anti-oxidant capacity, decreasing ROS production, and protecting cellular structures from UVR-induced damage (7).

A recent study described how 12-weeks of oral treatment with lycopene-rich tomato nutrient complex (TNC) inhibited the expression of UVB/A triggered genes that mediated skin's response to UV radiation (8). Lycopene inhibited UVA/B induced overexpression of heme oxygenase-1 (HO-1), an indicator of oxidative stress, and also decreased UVA/B induced overexpression MMP-1, a metalloproteinase involved in the breakdown of collagen and skin photoaging. Finally, lycopene curbed the expression of the inflammatory mediator ICAM-1, suggesting that this agent can inhibit the recruitment of leukocytes to the skin upon UVR-mediated damage and inflammation (8). Another recent study has shown that treatment of Skh-1 mice for 34 weeks with tomato-rich diet significantly decreased tumor induction by UVB irradiation compared to animals receiving a regular food (15). Moreover, the combination of lycopene with other carotenoids and *Lactobacillus johnsonii* also protected against UVA-induced polymorphous light eruption in human subjects (14).

The three clinical trials referenced above (6–8) had in common the duration of the treatment (12 weeks). However, they used different concentrations of lycopene and/or supplements, e.g., olive oil. Hence, it is not possible to properly correlate the doses with the observed effects. The anti-oxidant power of lycopene is well-proven regarding photoprotection, but there is not a consensus regarding the preventive dose required and the effect of combining it with other substances, highlighting the need for additional clinical research in the use of lycopene for oral photoprotection.

Beta (β)-carotene is a compound often administered for systemic photoprotection. However, studies demonstrating a protective effect of oral treatment with β-carotene against skin photodamage are scarce or revealed contradictory results. Intervention studies showed that a high intake of β-carotene decreased UVR induced erythema, but the efficacy of β-carotene depended on the dose and duration of treatment (31). Healthy volunteers receiving a supplement of β-carotene exhibited a slight increase of the threshold of minimal erythema dose (MED) (4). Similarly, partial protection against UVA and UVB radiation were observed in a study in which β-carotene was administered orally (5). Specifically, β-carotene reduced serum lipid peroxidation in a dose-dependent manner (5).

Regarding the effect of β-carotene in UVR-induced erythema, a placebo-controlled study showed that pretreatment with β-carotene diminished the intensity of erythema caused by sunlight (34). Similarly, oral administration of β-carotene in volunteers with Fitzpatrick's skin phototype II decreased the severity of UVR-induced erythema (35). Thus, in the supplemented group with β-carotene, Δα-values significantly decreased by 37.3% after 12 weeks of treatment compared to untreated group (35). In all the studies that documented some protection against UVR-induced erythema, the period for the supplementation was

TABLE 1 | Photoprotective effects of vitamins and their molecular targets.

UV effects tissue/ cellular/molecular target	Compound(s)	Results	Models	References
Erythema	β-carotene	Slight increase in MED/ *Min. protection/≥10 weeks doses high ≥12 mg carotenoids/ day*	Human	(4, 5)
	Lycopene	Decreases erythema	Human	(6, 7)
Oxidative stress	Lycopene	Inhibits HO-1	Human	(8)
	Astaxanthin	Inhibits reductions SOD, GSH	*in vitro*	(9)
DNA damage	Lycopene	Inhibits mtDNA deletion	Human	(7)
Inflammation	Lycopene	Inhibits ICAM-1 expression	Human	(8)
	Astaxanthin	Inhibits MIF, IL-1β, TNF-α expression	*in vitro*	(10)
		Decreases masts cells	Mice	(11)
		Sustains trans-UCA levels	Mice	(10)
	Lutein/Zeaxanthin	Suppresses skin edema	Mice	(12)
		Decreases masts cells number	Mice	(13)
	Lycopene β carotene, *Lactobacillus Johnsonii*	Inhibits PMLE	Human	(14)
Immuno-suppression		–	–	–
Photo carcinogenesis	Lycopene	Inhibits skin tumor formation	Mice	(15)
	Astaxanthin	Inhibits apoptosis	*in vitro*	(10)
	Lutein/Zeaxanthin	Decreases BrdU + epidermal cells	Mice	(12)
		Decreases PCNA + epidermal cells	Mice	(12)
		Increases tumor-free survival time	Mice	(13)
		Inhibits tumor volume and multiplicity	Mice	(13)
UV-ECM damage	Lycopene	Inhibits MMP-1	Human	(7, 8)
	Lutein/Zeaxanthin	Inhibits MMP-1, MMP-7. Stimulate TIMP-2	*in vitro*	(16)
		Inhibits MMP-13	Mice	(10)
		Decreases overexpression of HO-1, ICAM-1, MMP1 genes	Human	(8)
	Lycopene, β-carotene, *Lactobacillus johnsonii*	Inhibits MMP-1	Human	(14)
UV effects tissue/ cellular/molecular target	**Interventions**	**Results**	**Models**	**References**
Erythema	Vit. E+ Carotenoids	Suppression/decrease erythema	Human	(17, 18)
	Vit. C+Vit. E	Increases MED	Human	(19)
Oxidative stress	Vit E+ Carotenoids	Decreases levels of lipoperoxide	Human	(18)
DNA damage	Nicotinamide	Prevents depletion of cellular NAD+	*in vitro*	(20)
		Inhibits PARP-1	*in vitro*	(20)
		Inhibits CPD and 8oxoG	Human	(21)
			in vitro	(22)
	Vit. D	Reduces thymine dimers	Human	(23)
			Mice	(24)
		Inhibits CPD	*in vitro*	(25)
Inflammation	Vit. D	Inhibits NO products	*in vitro*	(23)
		Decreases edema and epidermal vesiculation	Human	(26)

(Continued)

TABLE 1 | Continued

UV effects tissue/ cellular/molecular target	Interventions	Results	Models	References
		Decreases TNF-α and iNOS	Human	(26)
		Induces overexpression of ARG1 and genes involved in skin repair	Human	(26)
Immuno-suppression	Nicotinamide	Prevents suppression of Mantoux reactions	Human	(27)
	Vit. D	Reduces CHS response	Mice	(24)
Photo carcinogenesis	Nicotinamide	Reduces AK by 29%	Human	(21)
		Lowers the rate of new NMSC and AK	Human	(28)
	Vit. D	Increases keratinocyte survival	*in vitro*	(23)
		Increases p53 expression	*in vitro*	(23)
UV-ECM damage		–	–	–

CHS, contact hypersensitivity; HO-1, Heme oxygenase-1; MED, minimal erythema dose; PMLE, polymorphic light eruption.

relatively long (∼≥10 weeks) with high doses (∼≥12 mg/day) (4, 5). This fact has raised concerns regarding the safety of administering such high doses of β-carotene. An epidemiological study suggested that high levels of β-carotene may have a deleterious effect in individuals at high risk of lung cancer, e.g., in smokers (more than a pack a day for 35 years) and asbestos workers. In these high-risk subjects β-carotene intake resulted in an enhanced risk of lung cancer compared to subjects bearing a lower risk of lung cancer (36). In a recent *in vitro* study published in 2018, the group of Lohan et al. has measured the anti-oxidant activity of β-carotene in a keratinocyte cell line (HaCaT) using electronic paramagnetic resonance spectroscopy and found that the anti-oxidant protection against UVR was achieved only with low doses of β-carotene whereas high doses were prooxidant (37).

However, based on long-term experience from the results obtained in the 1970s (4) and controlled trials (38) oral administration of β-carotene has been the treatment of choice to improve the photosensitivity of patients with erythropoietic protoporphyria. Photosensitivity was reported being reduced in ∼80% of patients to allow them normal life activities (38). The doses recommended range from 30 to 90 mg/day for children and 60–180 mg/day for adults, to reach a maximum plasma level of 600–800 μg/dl. More recently, subcutaneous administration of afamelanotide, an analog of the α-melanocyte-stimulating hormone, that darkens the skin, has been proposed as a novel treatment for erythropoietic protoporphyria (39).

Xanthophylls

Xanthophylls include some other carotenoids, e.g., lutein, astaxanthin, and zeaxanthin, which all have been shown to prevent photodamage induce by sunlight (9).

Astaxanthin

Astaxanthin is a non-provitamin A carotenoid mainly found in fish and shellfish (10). It is endowed with an anti-oxidant effect more potent than other carotenoids, including β-carotene and exerts anti-oxidant benefits without having prooxidant side effects. Astaxanthin inhibits the production of lipid peroxides induced by UVA. *in vitro* experiments have indicated its anti-oxidant and anti-inflammatory activity. In human skin fibroblasts astaxanthin prevented UVA-induced alterations of superoxide dismutase (SOD) activity and the anti-oxidant glutathione (GSH) (40).

Furthermore, treatment with astaxanthin reduced UVB- or UVC-induced expression of macrophage migration inhibitory factor (MIF), interleukin-1 (β IL-1β), and tumor necrosis factor α (TNF-α) (41). Astaxanthin significantly inhibited UV-irradiation-induced apoptosis in HaCaT keratinocytes (10). Treatment with astaxanthin before and after irradiation with UVB and UVA (41) decreased MMP-1 expression (11). Also, astaxanthin inhibited the UVB-induced expression of activator protein AP-1 and reduced UVB-induced phosphorylation of several MAPK family members via AP-1 transactivation in human fibroblasts (11).

A recent study reported the beneficial effects of oral astaxanthin on skin photoaging prevention *in vivo* (10). In a mouse model, astaxanthin inhibited the UVA-induced decrease of pyroglutamic acid (PCA) and urocanic acid (UCA), which are the primary natural moisturizing factors in the epidermis (10). In this murine model, astaxanthin also inhibited UVA-induced expression of matrix metalloprotease 13 (MMP-13), which may underline its photoprotective effect against skin photodamage (10).

Beneficial effects of astaxanthin have been reported with regard to human skin aging by Chung et al. (42). The same group of investigators is conducting a clinical trial to determine the effects of supplementation with astaxanthin or isoflavone on skin elasticity, epidermal hydration, and changes the skin barrier integrity. However, the results of this study are yet to become available.

Lutein and zeaxanthin

The xanthophylls, zeaxanthin, and lutein stand for 20–30% of the total carotenoids present in human serum and 80–90% of the carotenoids in the human retina.

Zeaxanthin is equitably distributed among plants, accompanying other carotenoids. It is typical of corn (maize), and also many bacteria produce it. Lutein is found in many vegetables, such as green beans, spinach, or broccoli, although its color is masked by chlorophyll. Zeaxanthin and lutein and are found in the macula where they contribute to preventing macular degeneration (43).

Lutein also accumulates in the skin. Its anti-aging and anti-carcinogenic properties are based on its anti-oxidants and anti-inflammatory effects against UVR damage. In mice, dietary lutein supplementation decreased ROS generation following UVR exposure (44). Specifically, our group reported the beneficial effects of orally administered lutein and zeaxanthin against the deleterious effects of UVB radiation. In hairless SKh-1 mice, supplementation with 0.4% lutein plus 0.04% zeaxanthin decreased the UVB-induced acute inflammatory responses (12). These photoprotective effects also included lower numbers of bromodeoxyuridine and proliferating cell nuclear antigen (PCNA)-positive cells in the epidermis, reduced skinfold thickness, and lower number of mast cells in the skin following UVB irradiation (13). Regarding UVR induced photocarcinogenesis, we found that oral supplementation with lutein/zeaxanthin significantly increased tumor-free survival time, decreased the total tumor volume, and reduced tumor multiplicity in comparison with control animals (13). We also reported lutein's photoprotective effects in UV irradiated dermal fibroblasts and melanoma cells. Lutein improved membrane integrity, increased cell viability, and decreased elastin expression. Lutein also inhibited UVR-induced overexpression of MMP-1 and MMP-2 while stimulating the endogenous tissue metalloproteinase inhibitor TIMP-2 (16).

Recently a placebo-controlled, double-blinded, randomized, crossover study reported that orally supplemented lutein caused a significant reduction of the overexpression of HO and MMP-1 genes induced by UVA radiation (8). Since these genes are reliable indicators of oxidative stress and photoaging, these results suggest that lutein may protect against photodamage produced by solar radiation (8, 45).

Nicotinamide

Nicotinamide is an amide form of vitamin B3 and a precursor of the essential coenzymes such as nicotinamide adenine dinucleotide (NAD^+) (21). Its primary dietary sources are liver, meats, yeast, legumes, nuts, green leafy vegetables, cereals, tea, and coffee (2, 21). It has been used to treat a variety of dermatological diseases such as atopic dermatitis and acne (2). Recent studies highlighted the role of nicotinamide, administered both orally and topically, as a chemopreventive agent against skin cancer. Its anti-cancer function is due to its corrective action toward UVR-induced DNA damage, also preventing immunosuppression.

Nicotinamide promotes genomic stability and DNA repair. NAD^+ is a substrate for poly-ADP-ribose polymerase 1 (PARP-1), which detects DNA damage (21). Nicotinamide prevents the depletion of cellular NAD^+ levels in response to exposure to UVR (20). Therefore, nicotinamide supplementation may prevent the progression of actinic keratosis (AK) to malignant squamous cell carcinoma (27, 46–48). In a very recent study, we found that niacin and its derivatives significantly promoted the expression of elastin, fibrillin-1, and fibrillin-2 in non-irradiated, and UVA-radiated fibroblasts, and directly inhibited MMP or elastase activity (49).

Nicotinamide also prevents UVR-induced intracellular depletion of adenosine triphosphate boosting cellular energy and enhancing DNA repair in HaCaT cells (20). In human, exposure to UV solar-simulated radiation triggered the formation of cyclobutane pyrimidine dimers (CPDs) and 8-oxo-7,8-dihydroguanine (8oxoG). Nicotinamide reduced CPDs and 8oxoG formation both *in vivo* and *in vitro* (22, 48).

Nicotinamide also inhibits the activity of sirtuins, which are NAD^+ dependent enzymes. Sirtuins play a mandatory role in cellular responses to environmental stress (47). Its effect on various transcription factors, including p53, contributes to the regulation of cell survival. Sirtuin expression is triggered by UV irradiation and is upregulated in AK and squamous cell carcinoma, suggesting that sirtuins may be associated with early stages of skin cancer. In healthy volunteers, using the Mantoux model of skin immunity, oral nicotinamide significantly reduced UVR-induced immunosuppression (27).

A potential protective role of nicotinamide in photocarcinogenesis has been reported in non-melanoma skin cancer (NMSC). In two clinical trials, nicotinamide decreased the incidence of NMSC and AK (28, 50). Immune-competent volunteers with $\geq$4 palpable AKs (face, scalp, and upper limbs) were treated with 500 mg of nicotinamide once a day for 4 months. Nicotinamide resulted in a relative reduction of 29% in AK count in the active treatment group compared with the placebo group (50). Along the same line, a double-blind, phase III controlled trial revealed that patients have suffered two or more NMSC and treated with nicotinamide had 23% lower rates of new NMSC and 11% less actinic keratoses than placebo-treated patients (28). This chemopreventive effect only persisted with continuous treatment (28). Adverse effects of nicotinamide were rare, and unlike niacin, nicotinamide is not a vasodilator. The administered dose of nicotinamide was 500 mg twice daily, and no more significant benefits were observed with higher doses (28).

However, a controversy emerged in response to a publication by Yelamos et al. (51). The authors concluded that nicotinamide may reduce the number of AKs, but only the less aggressive types, whereas in overall it may increase the rate of more aggressive types. The effect of oral nicotinamide as a chemopreventive agent against skin cancer may be due to its ability to enhance DNA repair and prevention of photoimmunosuppression (52). Additional clinical trials with larger cohorts of patients and more extended follow-up periods are necessary to solve this apparent controversy.

Vitamin D

Vitamin D3 (cholecalciferol) is obtained mainly from two essential sources: diet (10%) and endogenous production by photochemical conversion from 7-dehydrocholesterol in the epidermis (90%). Endogenous synthesis is induced by exposure of the skin to ultraviolet B (UVB) radiation. The skin is also a

target tissue for the active form of Vitamin D3 [calcitriol, 1,25 (OH) 2D3] and other biologically active metabolites of vitamin D3 (53). 25-hydroxyvitamin D_3 and 1,25-dihydroxy vitamin D_3 are also produced by keratinocytes and macrophages (54, 55). Vitamin D3 modulates inflammatory, immune responses and carcinogenesis (56, 57). Vitamin D3 decreases the inflammatory response by negatively regulating pro-inflammatory mediators, including TNF-α and nuclear factor-κB (NF-κb) one of the essential factors in inflammation. Vitamin D3 also decreases cyclooxygenase 2 (COX2), with the consequent decrease in prostaglandin levels (56, 58). The action of this vitamin has been reported in a mouse model of chemically-induced skin injury where a single dose of it attenuated the inflammatory response by inhibition of iNOS protein (or *NOS2)* gene and TNF-α protein (or *TNFA* gene) (59).

Similar to many other steroid hormones, 1,25 (OH) 2D3 exerts its action primarily through two signal transduction pathways: the classical genomic and the non-genomic pathway. The non-genomic effects depend on the levels of intracellular calcium whereas the genomic effects are mediated by the vitamin D receptor (VDR) (56). Recent findings support the role of VDR as a tumor suppressor in the skin. The anti-tumor effects of VDR are mediated, at least in part, by its interaction with p53 gene in response to UVR-induced DNA damage. Several studies have proposed that vitamin D3 also regulated the Hedgehog (Hh) signaling pathway. The Hh signaling pathway has been related to basal cell carcinomas (60). In the skin, keratinocytes, melanocytes, fibroblasts, and Langerhans cells express the VDR (53).

In vitro and *in vivo* studies showed that treatment with 1,25(OH)2D3 increased the survival of keratinocytes post-UVR compared to vehicle (23, 25). 1,25(OH)2D3 caused a significant reduction in the formation of CPD (23, 25) and increased the expression of p53 in keratinocytes (23). Moreover, dietary supplementation of 25(OH)D3 reduced UVB mediated contact hypersensitivity (CHS) response in C57BL/6 mice, a murine model with high susceptibility to UVB-induced systemic immunosuppression compared to mice with a deficient diet of this compound. Similarly, there was also a reduction in CPDs and inflammation in the animals supplemented with 25(OH)D3 (24).

In a recent clinical trial, participants were treated with a single oral dose of vitamin D3 (cholecalciferol) 1 h after UVR exposure. After irradiation, the human skin showed histological damage, including edema formation and epidermal vesiculation, which was diminished in a vitamin D3 dose-dependent manner. Skin expression of TNF-α and inducible isoform of nitric oxide synthase (iNOS) was lower in participants receiving Vitamin D3 as compared in those receiving placebo (26). In the same study, the genetic profile of the participants was evaluated independently of the treatment. Two distinct groups were identified. Group 1 was characterized by a lower expression of arginase (ARG)-1, which favors tissue repair and inhibits inflammation. Group 2 was marked by overexpression of ARG1 and genes involved in the restoration of the skin barrier. When assessing the treatments given in both groups, it was found that in group 2 all the participants had received a high dose of vitamin D3 and no participant received placebo. As a result, most participants in group 1 received placebo, and some received different doses of vitamin D3. Group 2 was identified as vitamin D3 responders of and group 1 vitamin D3 non-responders. The Vitamin D3 non-responders (group 1) had overexpression of proinflammatory genes, for example, IL-1α, MMP-1, and MMP3. In contrast, vitamin D3 responders (group 2) did not exhibit this characteristic. Similarly, IL-6 was activated significantly in patients who did not respond to vitamin D3. The authors of this trial proposed that a single oral dose of vitamin D3 rapidly mitigated the local UVR-induced inflammatory response in sensitive individuals. They also found that vitamin D3 responder showed a marked decrease in facial redness after an experimental sunburn, less evidence of epidermal damage and a lower expression of proinflammatory markers in the skin. As outlined above, the vitamin D3 responders had a genetic profile of overexpression of cutaneous barrier repair genes. Since the dose of vitamin D used had no adverse effects, and the calcium levels remained normal, the investigators of the study concluded that a single dose of high vitamin D3 could be of clinical use to prevent photodamage (26). Growing evidence sustains the perception that vitamin D pathway is relevant for photocarcinogenesis and that the pharmacological action of vitamin D, 1,25 (OH)2D3 and its analogs represent an advantageous new strategy for the prevention of UVR-induced damage (26, 61).

Vitamin C

Vitamin C given alone does not prevent the deleterious effects of UVR in the skin (19). Consequently, dietary supplementation of vitamin C (500 mg/day) for 8 weeks did not affect the UVR-induced erythema response. Furthermore, vitamin C supplementation in this group of healthy volunteers produced a paradoxical effect since the content of malonaldehyde and thiol-containing, and glutathione-binding proteins were reduced in the skin (62).

Vitamin E

Skin exposure to UVR depleted the cutaneous levels of vitamin E (alpha-tocopherol), implying that vitamin E is efficiently quenching ROS in UVR skin exposure (63). However, there is no evidence about the beneficial effects of oral vitamin E supplementation in the reduction of UVR-induced skin damage (19, 64). Conversely, supplementation of other components with vitamin E does show some benefit (see below) (19, 65). Likewise, MED was not changed by 400 IU of oral vitamin E alone after administration of 1 and 6 months (64). A side-by-side comparison of the effects of β-carotene (15 mg/day) vs. vitamin E (400 IU/day) for 8 weeks revealed that only vitamin E decreased the skin malondialdehyde concentration. However, neither β-carotene nor vitamin E changed other measures of oxidation UVR-exposed skin (65).

Vitamin Mixtures (Table 1)

Several groups or researchers from pharmaceutical companies and academic institutions developed mixtures of anti-oxidant. Such combinations were found to possess slight photoprotective, but they need to be administered at high doses and for an

extended period of time to obtain a modest degree of protection (17).

Carotenoids and Vitamin E

Supplementation with carotenoids and vitamin E for 3 months provided minimal photoprotection. Erythema was diminished with carotenoids (decreased of Δα erythema values by 34.5% after 8 weeks of treatment), but erythema suppression was amplified by the combination of carotenoids and vitamin E (decreased Δα-values by 43.19%) (66). An anti-oxidant complex with vitamin E with b-carotene and lycopene (with additional selenium and RRR-a-tocopherol) also protected against UVR-induced skin damage (18). This anti-oxidant compound increased the actinic erythema threshold, increasing MED by 20%. The anti-oxidant complex also decreases the p53 expression and lipoperoxide levels (18).

On the other hand, mixtures of anti-oxidants containing carotenoids (b-carotene and lycopene), vitamins C and E, selenium, and proanthocyanidins revealed no significant change in light sensitivity. However, they showed a decrease in UVR-dependent expression of MMP-1 (67).

Vitamin C and Vitamin E

Based on the rationale that supplementation of vitamin C regenerates cutaneous vitamin E from its radical form, the combination of both was thought to act synergistically. In this regard, different studies investigating supplementation with a mixture of vitamin C and vitamin E has been reported. In a retrospective human study, the combination of ascorbic and a-tocopherol during 7 weeks increased the MED by 77.6% (from 103 ± 29 mJ/cm^2 before supplementation to 183 ± 35 mJ/cm^2) (19). Similarly, during 1-week of oral intake of C and vitamin E increased protection of skin against UVR, as it increased MED by 21% (68). In another study, the same group of investigators studied the administration of ascorbic acid and α-tocopherol over a period of 12 weeks, which increased MED by 41% and decreased UVR-induced CPD (69). In another study of the same group of investigators ascorbic acid and α-tocopherol were given over a period of 12 weeks, and they found an increase of the MED by 41% and decrease of UVR-induced CPD (72). The addition of 3-methoxy-4-hydroxycinnamic acid (ferulic acid) did improve the stability of the combination of both vitamins. 1% alpha-tocopherol and vitamins (C+E) provided doubled photoprotection to solar-simulated radiation of skin as measured by both sunburn cell formation and erythema. Inhibition of apoptosis with the combination of both vitamins and ferulic acid was associated with inhibition of UVR-induction of caspase-3 and caspase-7 (70). The mechanism of this synergy does not seem to be clear, but it could be due to the power of ascorbate to produce a reduction of tocopherol, by transferring free radicals captured to the medium. On the skin, these free radicals are neutralized by other anti-oxidant systems.

DIETARY NON-BOTANICALS (TABLE 2)

ω-3 Polyunsaturated Fatty Acids

Omega-3 polyunsaturated fatty acids have been considered to treat skin conditions related to UVR exposure. They modestly decreased the appearance of sunburn cells and inflammation upon UVR treatment as well as long-term effects of UVA exposure (75). Omega-3 fatty acids were effective in the treatment of Hydroa vacciniforme (HV), a rare photodermatosis (76). Their main limitation as an oral photoprotector is that a relatively high dose is needed for the effect, often being higher than the gastric tolerance threshold. Another drawback is their unpleasant taste.

TABLE 2 | Photoprotective effects of non-botanical compounds and their molecular targets.

UV effects tissue/ cellular/molecular target	Compound(s)	Results	Models	References
Erythema		–	–	–
DNA damage		–	–	–
Inflammation	**Probiotics** *Lactobacillus johnsonii* (La1)	Increases IL-10	Mice	(71)
Immuno-suppression	**Probiotics**			
	La1	Suppresses CHS reaction	Mice	(71)
	Lactobacillus rhamnosus GG	Increases number of activated dendritic cells in the mesenteric lymph nodes	Mice	(72)
	La1	Facilitates recovery of eLc	Human	(73)
	La1+carotene	β-decreases PMLE	Human	(74)
	La1+carotene	β-decreases CD45+ dermal inflammatory cells.	Human	(74)
Photo carcinogenesis	**Probiotics**			
	Lactobacillus rhamnosus GG	Delays appearance of skin tumors	Mice	(72)
UV-ECM damage		–	–	–

eLc, epidermal Langerhans cells. EPP, erythropoietic protoporphyria; PMLE, polymorphic light eruption; CHS, contact hypersensitivity.

Probiotics

Probiotics are living microorganisms that regulate the immune system of the gut and defend it against inflammatory and infectious diseases.

In hairless Skh-1 mice exposed to UVR, supplementation with *L. johnsonii* NCC 533 (La1) conferred protection against the UVR-induced suppression of CHS and increased IL-10 serum levels (71). Oral administration of *Lactobacillus rhamnosus GG* delayed the onset of skin tumors in mice chronically irradiated with UV radiation. A significant improvement of the immune response was found in the small intestine of *Lactobacillus rhamnosus GG* treated mice with an increase of activated dendritic cells (72).

In humans, La1 supplementation accelerated the recovery of the function of Langerhans cell after UVR exposure in humans (73). Also a human dietary supplement combining La1 with nutritional doses of β-carotene prevented sunburn and sun intolerance in most of the study participants, protecting against the development of UVA-induced polymorphous light eruption (74).

The role of probiotics in photoprotection is promising, but it is necessary to carry out more extensive clinical trials before making a definitive recommendation on the use of probiotics as oral photoprotective agents (77).

Idebenone

Idebenone, a lipophilic coenzyme Q10 analog, has a relatively high penetration into the skin upon topical administration. Its efficacy as an oral photoprotector has not been studied, but its oral administration increased the expression of nerve growth factor (NGF), and it is beneficial in patients with Leber's hereditary optic neuropathy (78).

DIETARY BOTANICALS (TABLE 3)

This general term includes anti-oxidant and anti-inflammatory polyphenols found in vegetable foods. In the last decade, plenty of interest has emerged regarding the possible health benefits of polyphenols as anti-oxidants. The main classes of polyphenols are phenolic acids, flavonoids, stilbenes, and ligands. Flavonoids represent the most significant natural anti-oxidants present in dietary botanicals. Due to their chemical nature, which contains phenolic rings, they can absorb free radicals to form phenoxy radicals (1, 3). There are different subfamilies of flavonoids owing their chemical structure, which include flavanonols, aurones, isoflavones, flavonols, flavones, and anthocyanins. On the following pages, we summarize the main findings regarding several subclasses of polyphenols as oral photoprotective agents.

Green Tea Polyphenols (GTPs)

The primary anti-oxidant moiety of green tea (*Camellia sinensis*) is a mixture of polyphenols (frequently referred to as catechins or green tea polyphenols, GTPs). The major catechins of green tea are epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate (ECG) and epicatechin (EC), epigallocatechin (EGC). EGCG constitutes ∼40% of total GTPs at the source (green tea leaves) (117). Numerous studies have demonstrated that tea catechins are efficient scavengers of ROS. Besides their anti-oxidant activity, catechins exhibit a modulating effect on inflammatory and immunomodulation responses playing an essential role in host defense against tumor development and progression (117). Interestingly, green tea confers protection against skin cancer in mice induced by UVA and UVB radiation (118).

Following standard photocarcinogenesis protocols using hairless mice, oral administration of GTPs in drinking water resulted in significant protection against the development of NMSC regarding tumor multiplicity, tumor size, and tumor incidence (percentage of mice with tumors) compared to no-GTPs-treated UVB-irradiated mice (109). Also, hairless mice receiving oral GTPs reduced UVB-induced overexpression of MMP-2 MMP-9 and enhanced expression of tissue inhibitor of MMPs. Oral GTPs administration also reduced UVB-induced expressions of vascular endothelial growth factor (VEGF), CD31 and inhibited expression of PCNA, resulting in decreased apoptosis and lower activation of the mitogen-activated protein kinase (MAPK) pathway (119).

GTPs also act against photoaging by preventing of UVR-induced activation of inflammatory transcription factors AP-1 and NF-κB (98, 120). When added in cultured human keratinocytes before, and/or after UVB irradiation, EGCG inhibited AP-1 activity (121).

It is precisely established that IL-12 deficiency increases the UVR-induced inflammatory response and decreases DNA repair in response to UVR-induced damage (91). In keratinocytes and human living skin equivalent models, GTPs induced the secretion of IL-12 and decreased keratinocyte apoptosis caused by UVB radiation (97). GTPs in drinking water significantly reduced the UVB-induced tumor development (volume and number of the tumors) and the number of CPD^+ cells in wild-type mice but did not affect IL-12-deficient mice (91). These data suggest that GTPs prevent the photocarcinogenesis primarily by a mechanism that involves IL-12.

GTPs also increase the expression of nucleotide excision repair (NER) genes. Oral GTPs in mice had in the skin a reduced the number of CPD^+ cells, showing thus faster repair of UVR-induced DNA damage. Also, GTPs decreased the migration of CPD^+ cells to draining lymph nodes (92). Moreover, green tea catechins (GTC) reduced UVR-induced inflammation and protected from UVR-radiation immunosuppression and photo-carcinogenic effects in rodent models, but human studies are scarce and controversial.

Rhodes and colleagues examined the ability of GTPs to protect the skin from the effects of UVR. Sixteen healthy human subjects were given GTPs in combination with a vitamin complex. The preparation reduced UVR-induced erythema and inhibited UVR-mediated up-regulation of pro-inflammatory metabolites produced by 12-lipoxygenase (12-LOX). 12/15-LOX enzymatic balance plays a role in the pathogenesis of skin disorders as it regulates cell proliferation and apoptosis. The investigators concluded that the intake of GTPs resulted in the incorporation of catechin metabolites to human skin associated with a decrease of the 12-LOXE metabolite, possibly promoting protection against inflammation from sunburn and damage mediated by UVR (79). However, in a more recent

TABLE 3 | Photoprotective effects of Botanical compounds and their molecular targets.

UV Effects Tissue/ cellular/molecular target	Compound(s)	Results	Models	References
Erythema	Green tea	Decrease erythema	Human	(79)
	Polyphenols	*Green tea catechins + Vitamin C*		
	Cocoa extract	Decreases erythema/Increases MED	Human	(80, 81)
	PL	Decreases erythema/Increases MED	Human	(82, 83)
				(84)
	Citrus + Rosemary	Increases MED	Human	(85, 86)
Oxidative stress	PL	Inhibits lipid peroxidation	Human *in vitro*	(87, 88)
		Enhances anti-oxidant plasma capacity	Mice	(89)
	Pomegranate	Inhibits lipid peroxidation Inhibits hydrogen peroxide	Mice	(90)
DNA damage	Green tea polyphenols	Decrease CPD	Mice	(91, 92)
		Increase NER genes	Mice	(92)
	PL	Reduces 8oxoG	Mice	(93)
		Reduces number of DNA mutations	Mice	(93)
		Inhibits CPD	Mice	(93)
			Human	(83, 94)
		Reduces common mitochondrial deletions	Human	(95)
	Pomegranate	Reduces 8oxoG	Mice	(90)
		Inhibits CPD	Mice	(90)
	Forskolin	Improves NER	*in vitro*	(96)
Inflammation	Green tea polyphenols	Induce the secretion of IL-12	*in vitro*	(97)
		Inhibit AP-1 NF-κB	Mice	(98)
		Inhibit 12-LOXE metabolites	Human	(79)
	PL	Inhibits TNF-α, iNOS, AP-1 NF-κB expression	*in vitro*	(99)
		Increases IL-10 expression	*in vitro*	(100)
		Inhibits leukocyte extravasation	Mice	(101)
		Decreases neutrophil and macrophages	Mice	(93)
		Decreases mast cells	Human	(83, 102)
		Inhibits COX-2, PGE2	Mice	(93)
			Human	(94)
	Pomegranate	Inhibits COX2, NF-κB;	Mice	(90)
Immuno-suppression	PL	Inhibits *trans*-UCA isomerization	*in vitro*	(103)
		Inhibits glutathione oxidation	Mice	(101, 104)
		Prevents eLC depletion	Mice	(101)
			Human	(82, 83, 105)
		Reduces PMLE reaction Improves subjective symptoms of PMLE	Human	(106, 107)
				(108)
Photo carcinogenesis	Green tea Polyphenols	Decrease keratinocyte apoptosis	*in vitro*	(97)
		Protect against the development of NMSC (tumor incidence, tumor multiplicity, tumor size)	Mice	(109)
		Reduce CD31 and VEGF expression	Mice	(109)
		Reduce tumor development (number of tumors, tumor volume)	Mice	(91)
		Inhibit PCNA + epidermal cells	Mice	(109)
	PL	Increases the number of p53(+) cells	Mice	(89, 93)
		Delays skin tumor development	Mice	(89)

(Continued)

TABLE 3 | Continued

UV Effects Tissue/ cellular/molecular target	Compound(s)	Results	Models	References
		Increases the clearance of AKs Decreases the recurrence rate of AKs	Human	(110)
		Increases MED in familial MM	Human	(84)
		Inhibits epidermalcell proliferation	Human	(83, 94)
		Decreases PCNA, Cyclin D1 expression	Human	(94)
	Isoflavones (Genistein)	Inhibit skin tumor formation	Mice	(111)
	Pomegranate	Inhibits PCNA expression	Mice	(90)
	Resveratrol	Inhibits NF-kB expression	*in vitro*	(96)
		Inhibits TGF-beta expression	Mice	(112)
		Decreases tumorigenesis	Mice	(112)
	Forskolin	Reduces sunburn cells	*in vitro*	(113)
UV-ECM DAMAGE	Green tea polyphenols	Reduce MMP-2 MMP-9 Enhance TIMP	Mice	(109)
	Cocoa extract	Attenuates skin wrinkling Decreases cathepsin G Improves Serpin B6c	Mice	(114)
	PL	Increases types I, III, and V collagen	*in vitro*	(115)
		Inhibits MMP-1	*in vitro*	(88, 115)
		Increases TIMP	*in vitro*	(115)
			Mice	(93)
		Decreases MMP1 after VIS-IR radiation	Human	(116)

NER, Nucleotide excision repair.

human study (clinicaltrials.gov, NCT01032031) from the same group of investigators (122) using equal oral doses GTCs and vitamin C during the same period, no significant reduction in skin erythema, or leukocyte infiltration was found. Also, the investigators did not see alterations in the eicosanoid response to UVR.

Together with the controversial human results, there are significant limitations for the widespread use of GTPs preparations in preventing photodamage and photocarcinogenesis. GTPs are very sensitive to oxidation, rapidly losing their activity. Their half-life in the bloodstream is <3 h (123). Another limitation is their poor solubility in lipid preparations, which significantly decreases its penetration through the skin, whereas it favors its absorption and oral uptake. To improve its penetration into the skin and its stability, GTPs can be mixed with non-toxic organic solvents, for example, oleic acid. However, it is necessary to further investigate the toxicity of GTPs at high doses (124).

Cocoa Extract

Cocoa (Chocolate) extracts are rich in polyphenols, mainly flavanols. Cocoa flavanols (CFs) have anti-oxidant properties, increasing the expression of HO-1 through of nuclear factor erythroid 2-related factor 2 (Nrf2) (125). Nrf2 is a regulator of cellular anti-oxidant responses that control the expression of genes encoding detoxifying proteins and anti-oxidant, such as HO-1. Cocoa procyanidins also inhibit MAPK activation and MMP expression (126). These mechanisms underlie their potential use in photoprotection and photocarcinogenesis (80, 125)*in vivo* studies showed that supplementation with cocoa powder in female albino hairless mice (Skh-1) attenuated UVB-induced skin wrinkling formation, regulating genes involved in extracellular dermal matrix degradation. Dietary cocoa decreased the expression of cathepsin G and improved the expression of Serpin B6c decreasing extracellular matrix (ECM) degradation (114).

In humans, oral consumption of CFs has potent anti-inflammatory, anti-oxidant, and photoprotective effects. In a clinical trial, two groups of healthy women, with Fitzpatrick's skin phototype II, undertook diets bearing high or low CFs for 12 weeks. A dietary beverage with cocoa rich in CF decreased the degree of erythema following irradiation with a solar light simulator ($\Delta\alpha$ valued decreased 68% from baseline). UV sensitivity did not change in the women with treatment with cocoa beverage bearing low doses of CFs (80).

In 2009, a double-blind study in 30 healthy subjects showed that consumption of a chocolate rich in flavonoids (HF) could prevent certain harmful effects of UV radiation in human skin, while conventional chocolate (LF) did not have this effect. MED after 12 weeks of HF chocolate treatment more than doubled, while it remained unaffected in subjects taking LF chocolate (81).

Polypodium leucotomos Extract (Fernblock®)

Polypodium leucotomos (PL) is a fern of the Polypodiaceae family, native to Central and South America. PL has been

used in traditional medicine in those geographical areas for the treatment of skin conditions (1). A standardized aqueous extract of PL (PL/Fernblock®) made from leaves of the fern PL, rich in polyphenols, has been developed to exploit the photoprotective properties of ferns and to provide a steady phenolic content (87, 127). Our group has thoroughly investigated Fernblock® with regard to its anti-oxidant, anti-inflammatory, and immunomodulatory and tumor growth suppressive properties (1). Phenolic compounds identified in the aqueous extract Fernblock® are 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid (protocatechuic acid), 4-hydroxy-3-methoxybenzoic acid (vanillic acid), 3,4-dihydroxycinnamic acid (caffeic acid), 4-hydroxycinnamic acid (p-coumaric),3-methoxy-4-hydroxycinnamic acid (ferulic acid), 4-hydroxycinnamoyl-quinic acid, and five chlorogenic acid isomers (128).

Ferulic and caffeic acids are the most potent anti-oxidants present in PL. Their apparent permeability shown in the Caco-2 cell *in vitro* model was 70–100%, similar to human post-oral administration absorption (127).

This extract was marketed in Europe in the year 2000, both in topical and oral forms, and is currently available in more than 26 countries, including the U.S. as a dietary supplement since 2006 (129). Its mechanisms of action and its success in clinical trials, and the increased social interest in natural substances such as polyphenols, have placed PL as an interesting photoprotective and anti-oxidant option (130, 131).

PL increases the ability of the endogenous anti-oxidant system. PL neutralizes superoxide anions, hydroxyl radicals, and lipoperoxides produced in the skin after exposure to UV and visible radiation (87, 88, 104, 127). The most significant differences between this extract and conventional anti-oxidants refer to its capacity as a superoxide anion scavenger. The majority of traditional anti-oxidants such as vitamin C, E, carotenoids are good quenchers of singlet oxygen; however, PL also exhibits excellent anti-oxidant properties against superoxide anion (87). In *in vitro* studies our group found that this extract was an efficient quencher of superoxide anion, with ∼40 to 60% of the activity of SOD used as a positive control. Furthermore, it also inhibited lipid peroxidation (87, 88, 127). In addition to its anti-oxidant activity, PL shows promise in the prevention of photodamage and photocarcinogenesis because it enhances DNA repair and modulates the inflammatory and immune responses (1, 3, 129).

In the context of UVR-induced inflammation, our studies have revealed that orally administered PL prevented erythema in the UVR-treated human skin (82, 87). After oral administration of PL, the MED increased by 2.8 ± 0.59 fold (82). PL is also active on the skin as a photoprotector against PUVA-induced phototoxicity (83, 105). The basis of its anti-inflammatory properties could be its ability to abolish the expression of the TNF-α, iNOS (99), redox-sensitive transcriptional factors activator protein 1 (AP-1) and nuclear factor κB (NF-κB) (99). PL also decreases the expression of COX-2 and PGE2 (93). However, the effect of PL on AP-1 and NF-κB expression after exposure to solar simulated radiation (SSR) cannot be explained only by the anti-oxidant action of PL since treatment with a bona fide anti-oxidant does not decrease the expression of AP-1 and NF-κB in human keratinocytes subjected to SSR. *in vivo* experiments showed that COX-2 and PGE2 were overexpressed after exposure to UVR, but they both decreased in PL-fed mice (93). Other beneficial effects of oral PL included a decrease in UVR-induced infiltration of neutrophils and macrophages into the skin (93). Other studies showed a reduction in the levels of the inflammatory molecules, both in humans (83, 105) and in mice (101). These studies revealed an inhibition of mast cells and leukocyte extravasation in the irradiated area when PL is administered orally. These data complement *in vitro* studies using human PHA-stimulated peripheral blood mononuclear cells, which showed that PL decreased the production of IL-2, IFN-gamma, and TNF-alpha and completely inhibited the expression of the inflammatory cytokine IL-6. In the same experiments, the addition of PL increased IL-10 production (100). PL also inhibited apoptosis and cell death (89, 99) therefore preventing apoptosis/necrosis-triggered inflammation.

Moreover, orally administered PL inhibited UVR-mediated DNA damage and mutagenesis in humans and mice (83, 94, 99). PL exerted its effect by a double mechanism by preventing UV-induced accumulation of CPDs and reducing oxidative damage, with a reduction of 8-OH-dG. Also, even before UV irradiation oral PL decreased the levels of 8-OH-dG in a mouse model of Xeroderma pigmentosum ($Xpc^{+/-}$), suggesting that oral PL relieves constitutive oxidative DNA damage (103). In this model, we found that PL inhibited expression of COX2 and accelerated CPD removal. In this regard, cells containing CPDs were detected immediately after UVB in both groups of animals, vehicle-, and PL-fed mice, confirming similar initial UVB damage. However, by 72 h, 54 ± 5% CPDs remained in vehicle-fed mice compared to only 31 ± 5% in PL-fed mice. These data indicate that PL increases the repair capacity rather than preventing the formation of thymine dimers. Also, we found that PL prevented UVR-mediated pro-oxidative DNA damage by quantifying cells containing 8-OH-dG, particularly in skin sections 6–24 h after exposure and also reduced the mutational burden by ∼25% (93). Finally, oral PL decreased UVA-dependent mitochondrial DNA damage by reducing common deletions (CD) (95).

Regarding photo-immunosuppression PL is endowed with immunomodulatory properties acting as a photoimmunoprotective agent by different mechanisms. PL prevents UCA isomerization into from its *trans* to the *cis* isomer (103), which is a triggering event of skin immunosuppression. In turn, as the primary UV-absorbing chromophore in the skin, it prevents the expression of pro-inflammatory cytokines such as TNF-α (99). Also, PL prevents epidermal Langerhans cells (eLC) depletion produced by UV irradiation *in vivo* (82, 83, 101, 105). Multiple molecular mechanisms may underlie the improvement of survival of dendritic cells, including inhibition of UCA isomerization, as mentioned above (103), blockade of iNOS expression (99), and improvement of endogenous systemic anti-oxidant systems (89, 93, 101). Finally, orally administered PL also inhibited UVB radiation-induced immunosuppression in mice sensitized with oxazolone before UVR exposure and prevented inhibition of CHS (102).

Photoinmunosuppression is an essential area for preventing photocarcinogenesis. Our group has evaluated the possible protective action of oral PL in photocarcinogenesis. *in vitro* and *in vivo* studies showed that PL modulates the expression of molecules, transcription factors, and gene expression involved in photocarcinogenesis (1–3, 132). We found that PL delayed the onset of skin cancer in PL-treated hairless mice. PL also decreased the number of precancerous lesion in the surrounding non-tumoral skin of the same animal and elevated p53 expression levels (89). The delay in the initiation of photocarcinogenesis correlates with changes in the levels of several markers of oxidative stress in the skin and blood.

In this regard, PL-treated animals had increased anti-oxidant plasma activity, without changes in the levels of endogenous anti-oxidant enzymes (89). Oral PL also induced p53 overexpression in the Xeroderma pigmentosum $Xpc^{+/-}$ mouse model that displays skin cancer highly comparable to mild human XP syndromes. PL-fed and UVB-irradiated, $Xpc^{+/-}$ mice showed a 2–4 fold increase in the levels of total and pSer15 compared to vehicle-treated mice (93). DNA damage induced phosphorylation of p53 on Ser15 and Ser20. Phosphorylation inhibited the ability of negative regulator of p53, to bind p53, favoring both the activation and accumulation of p53 in response to DNA damage (133). In this experimental model, we found an inverse correlation between the increase of p53 and the decreased COX-2 levels, suggesting that oral PL treatment reduced UVR-induced COX-2 levels, at least in part, by activation of p53 (93). In agreement with the increased p53 expression, PL also decreased epidermal cell proliferation induced by UVR in human and experimental animals (83, 104). In clinical studies, we found that PL reduced the rate of proliferating epidermal cells induced by UVR (83). A recent study showed that PL decreases the number of cyclin D1- and PCNA-positive epidermal cells caused by UVR (134).

The ECM provides structural integrity to the tissue and is remodeled during skin aging/photoaging and cancer (115). *in vitro* experiments showed that PL directly inhibited the enzymatic activity and expression of MMPs in melanoma cells and fibroblasts. PL stimulated the expression of TIMPs in melanoma cells, reducing melanoma cell growth, and ECM remodeling (88, 115).

VL and infrared radiation (IR) also promote sun-induced skin damage (135, 136). The energy of IR and VL photons is much lower than that of UV photons. The most considerable part of solar IR radiation is IRA (IRA, wavelength 700–1,400 nm). IRA deeply penetrates into the human skin whereas IR B (IRB, wavelength 1,400–3,000 nm) and infrared radiation C (IRC, wavelength 3,000 nm−1 mm) only affect the upper layers (135). In human skin, IR irradiation generates heat and free radicals (136). IRA-induced photoaging, by generating mitochondrial ROS (137) followed by a cascade of intracellular events that leads to an increase of MMP-1 and MMP 9 without an increase of TIMP expression (138). Besides its effect on MMP, IRA also triggers infiltration of inflammatory cells into the skin (139). IRA, also, decreases the number of Langerhans cells, influences wound repair and alters the expression of transforming growth factor beta (TGB-β) (139). Regarding VL (400–700 nm), an early study from Pathak (140) indicated that VL produced an immediate darkening of the skin. VL contributed to ROS production in the skin (141) and induced DNA damage through the generation of ROS (142). VL exerts similar effects to UVR in the ECM. IR plus VL increased the expression of MMP-1 and MMP-9 and, in human skin *in vivo* lowered type I procollagen levels and recruited macrophages to the irradiated site (139).

We have also studied the possible effect of PL in preventing damage induced by IR plus VL. We found that PL was clinically effective in preventing the deleterious effects of infrared-visible IR–VL radiations (116). In a recent prospective clinical trial, volunteers received a combination of IR-VL (600 and 200 J/cm^2, respectively). Gluteal biopsies were taken before and after irradiation. PL (960 mg/day) was administered orally for 21 days followed by another round of IR–VL radiation and biopsy. The results showed that MMP-1 was increased after VL-IR radiation concerning baseline in 71% of the patients, while the percentage of patients treated with PL was smaller (51%).

As we reported previously, PL reduces UVR-induced immunosuppression and mutagenesis. Patients with at least two AKs on the scalp underwent two sessions of PDT, separated by 1week. One group received PTD and oral PL treatment for 1 week after the last PDT session. Both treatment modalities PDT alone or PDT plus oral PL reduced the number of AK. However, supplementation with oral of PL increased the clearance rate and decreased the recurrence rate of AKs within 6 months, compared to PDT alone. Oral PL could be used as a supplementary agent in the treatment of field cancerization (110). We have also investigated the possible protective role of oral administration of PL in patients at risk of malignant melanoma (MM) and evaluated the influence of PL in the interaction between MC1R polymorphisms and the cyclin-dependent kinase (CDK) inhibitor 2A gene (*CDKN2A*) status with MED (84). 25–50% of familial MM relatives display a mutation in *CDKN2A* and variants in *MC1R* are common in the white population, conferring low to moderate risk to develop melanoma. In our trial, a total of 61 patients (25 with familial and/or multiple MM, 20 with sporadic MM, and 16 without a history of MM) were exposed to UVB radiation. Oral PL treatment increased by 30% the MED mean in all patients. Among patients with familial MM, those individuals with mutations in CDKN2A and/or MC1R had greater differences regarding the response to treatment with PL (84). According to these results, patients with higher UVR sensitivity (lower basal MED) would benefit the most with oral PL treatment. These results are intriguing and thus studies with longer-term PL administration in patients with a high risk of developing MM are needed to consolidate these data. Finally, PL also ameliorates the onset of the polymorphic light eruption, which is the most common photosensitivity condition of the skin (106–108).

Regarding the safety of oral treatment of PL, a recent study determined that capsules containing a carefully controlled extract of PL (Heliocare, IFC, Spain) (240 mg) have not produced severe adverse effects, after 2 months of treatment (143).

Isoflavones

Isoflavones, one leading group of phytoestrogens, have the ability to act as topical photoprotectors. Oral photoprotection is not well-documented (144), and also not much information has been

reported from studies in humans. Some isoflavones or isoflavone-rich compounds are genistein, equol, silymarin, quercetin, and apigenin.

Genistein

Genistein, an isoflavone obtained from fermented soy, coffee beans, and fava, is a potent tyrosine kinase inhibitor. Genistein has a robust anti-oxidant capability (145). Expression of the transcription factor Nrf2 is activated by oral treatment with genistein (146, 147). Oral genistein inhibited UVB-mediated skin photoaging and skin tumor formation in a rodent model (111).

Equol

Equol, a metabolite of the genistein analog daidzein, is enriched naturally with red clover (*Trifolium pratense*) (148). Although equol has yet to be used as an oral photo-protector, recent research indicates a high oral tolerance (149), suggesting that it may be appropriate for oral photoprotection. Topically, equol confered protection against photoaging (150) and also decreased tumorigenesis induced by UVR (149, 151).

Silymarin

Silymarin is a flavonoid derived from the milk thistle plant (Silybum marianum complex) that contains silybin, silydianin, and silychrisin. Its oral use in photoprotection has not been tested, whereas sylimarin topically applied confers photoprotection due to the amount of silybin in the preparation (152). Silymarin interferes with the bioavailability of other drugs (152) what may limit the use in oral photoprotection.

Quercetin

The polyphenol quercetin is the most abundant flavonoid, and it is found in fruits, vegetables, tea, and wine. Quercetin is a potent anti-oxidant, and it works as a topical photoprotector (153), but until now it has not been evaluated in oral photoprotection. Similar to silymarin, it can alter the bioavailability of other drugs (154).

Apigenin

Apigenin is a flavonoid found in several fruits, vegetables including onions, parsley, and sweet red peppers as well as tea. Several studies conducted over the past years have reported its potential as an anti-oxidant, anti-inflammatory, and anti-cancer compound (155). Topically, apigenin decreased tumor emergence after exposure to UVR in a rodent model. This effect may have been caused, at least in part, by inhibition of both COX2 and the mammalian target of rapamycin signaling pathway (156–158). However, its usefulness as an oral photoprotector has yet to be addressed.

Pomegranate (*Punica granatum*, fam. Punicaceae)

The anti-oxidant activity of pomegranate juice is very high, e.g., higher than that of red wine and green tea due to its polyphenolic content, which includes anthocyanidins and catechins and tannins (159). As an oral photoprotector, the Mukhtar group has described the efficacy of pomegranate polyphenols in the prevention of photocarcinogenesis in mice irradiated with UVB (90, 160, 161). These authors claimed that pomegranate fruit extract inhibited the expression of COX-2 and iNOS, as well as the expression of cyclin D1 in mouse skin after UVB irradiation. Also, this extract decreased the expression of MMP2, 3, and 9 in the skin of the mouse model (90, 160, 161).

Citrus Plus Rosemary Extract

Citrus contains a large amount of flavonoids, and rosemary is rich in polyphenols and diterpenes. In humans, oral administration of a combination of citrus and rosemary extracts decreased sensitivity to erythema induced by UVR, as quantified by an increased MED that after 8 weeks of treatment ranged from 34% in Perez-Sanchez's study (85) to 29.8% in Nobile's study (86).

Resveratrol

Resveratrol is a polyphenolic phytoalexin stilbenoid found in the peels and seeds of grapes as well as red wine. The effect of resveratrol as a topical photoprotector is well documented (162). Regarding its action as an oral photoprotector in a p53-sensitive mouse tumor model, the administration of oral resveratrol decreased the tumorigenesis mediated by UVR (112) through the modulation of TGF-beta (112) and NF-kB (163). Also, resveratrol may have the potential to stimulates the response to radiation therapies (164).

Forskolin

The diterpenoid forskolin (FSK) is obtained from the root cork of the Indian coleus (*Coleus forskohlii*). It is a classical activator of the adenylate cyclase enzyme resulting in elevated levels of cyclic adenosine monophosphate (cAMP). A recent study addressing the effect of FSK in UVR-mediated photodamage reported that FSK accelerated the removal rate of UVR-induced photolesions *in vitro* and *in vivo* (96). Topical application of forskolin also restored pigmentation UVR-independent in an MC1R-defective fair-skinned animal model (165).

Cutaneous melanocortin one receptor (MC1R) initiates multiple protective actions against deleterious effects of UVR, including melanin production. These actions are mediated by the activation of adenylyl cyclase and cAMP. Eumelanization by FSK is thought to occur by direct activation of adenylyl cyclase in melanocytes and up-regulation of melanocyte cAMP levels. Polymorphisms of MC1R induce a fair-skinned, sun-sensitive, and cancer-prone phenotype. Mice bearing inactivating mutations in this gene (Mc1re/e) lacked the ability to generate cAMP in response to MSH. In those mice, cutaneous application of FSK promoted DNA repair in response to UVR photodamage. The defect of these transgenic mice underlies in an inability to remove CPD induced by the UVR, which is significantly increased by FSK to levels comparable to those of Mc1r wild-type mice (166). FSK also exerted its photoprotective effect by increasing epithelial thickening due to increased keratinocyte proliferation in a cAMP-dependent manner (167). *in vitro*, FSK has also demonstrated a photoprotective impact by increasing epithelial thickness, favoring the proliferation of keratinocytes in a cAMP-dependent way (113). *in vitro*, FSK inhibited keratinocyte apoptosis induced by UVR, reducing sunburn cells count. Interestingly, melanin content levels were independent of

FSK treatment, showing that the protection against apoptosis was not the result of an increase in melanin levels (168).

FSK also promotes cellular growth to repair skin photodamage. Specifically, FSK improves NER after exposure to UVR; however, this effect only appeared in growing skin cells. When cells were cultured at low density, FSK stimulated cAMP responsive element binding (CREB) phosphorylation, which is a marker of PKA activation, producing a significant increase in the activity of NER compared to the control. These findings indicate that cell growth is critical for FSK to improve NER function and suggest that cell growth conditions should be considered as a variable while evaluating the FSK efficacy in inhibiting UVR-induced photodamage (96). FSK has been used orally for non-skin-related therapeutic uses, but not in skin disease (168).

EVALUATING ORAL PHOTOPROTECTION

The classical model of evaluation of topical photoprotectors includes SPF assessment, based on prevention of erythema. Another useful indicator is the erythema protection scale, which measures skin reddening due to inflammation. However, oral photoprotectors are not very effective in reducing erythema and thus cannot be evaluated using SPF and erythema protection factor scales. These reagents need to be measured according to other parameters, which include:

Anti-oxidant Activity

Approaches include irradiation of keratinocytes with UVB followed by detection of T-T dimers and sunburn cells and have the potential to become a gold standard to gauge the photoprotective ability of new oral compounds. An additional test could include measuring anti-oxidant potential *in vitro*. The main drawback is that this approach does not allow to extrapolate the effect of oral administration directly. In general, the previous methods always need to be complemented with studies on oral toxicity, metabolic disposition, and careful assessment of the pharmacodynamics and pharmacokinetics of an oral agent.

Anti-mutagenic Activity

This approach is currently applied in nonhuman models, and it is based on the ability of the compound(s) under analysis to prevent mutations in critical genes involved in photocarcinogenesis, e.g., p53 (169). Two common reference assays employ mouse bone marrow-derived erythrocytes and the TA100 strain of *Salmonella typhimurium*, which is histidine-dependent.

Photoimmunoprotection

A useful parameter includes measuring the effect of the oral intake of the compound of interest on UVR-induced inhibition of contact or delayed-type hypersensitivity responses. This measure can be done in one or two ways: (i) a single sub-erythemal dose of UV radiation. This protocol enables a more direct comparison with the SPF parameter used to evaluate topical sunscreens. However, this approach requires a large cohort of healthy volunteers. This renders this approach not particularly cost-effective (170); (ii) using a pre-sensitization screening with chemical irritants. A significant problem with this approach is that the chemical sensitization is not directly comparable to damage induced by UVR. However, it brings a reasonable estimate of the immunomodulatory properties of the treatment.

The practical aspects of the use and prescription of oral photoprotectors need to be evaluated by available information on biodisposition, efficiency, and safety. A gold standard is still lacking in this regard, but one positive is the overall low toxicity of these agents (after all, many of them are part of nutrients). However, specific aspects, e.g., known allergies, must be taken into account when using or prescribing these approaches.

FUTURE PERSPECTIVES

Oral supplementation aims at countering the long-term effects of sun exposure. Many of these effects are related to immunosuppression, chronic inflammation, and photocarcinogenesis. The current view of many research groups, including ours, is that this developing field needs the establishment of strong standards to enable a rigorous assessment of the effectiveness of oral photoprotection. These need to include measurements on anti-oxidant activity, anti-mutagenic capability, and anti-immunosuppressive function. The FDA, EMA, and other regulatory agencies around the world need to become involved in the establishment of gold standards and regulate the research on the growing landscape of new substances and combinations of substances that will likely change the field of photoprotection in years to come.

AUTHOR CONTRIBUTIONS

SG conceived the review and wrote the sections of *Evaluating Oral Photoprotection* and *Futures Perspectives.* CP, NP, YG, and AJ have contributed to the different sections of the manuscript and have read and corrected the entire manuscript. SG has overseen the integration of the entire manuscript and has read and corrected the entire manuscript.

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