Photochemical & Photobiological Sciences

PERSPECTIVE

Cite this: *Photochem. Photobiol. Sci.*, 2018, **17**, 1872

Ultraviolet radiation-induced immunosuppression and its relevance for skin carcinogenesis

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The realisation that UV radiation (UVR) exposure could induce a suppressed immune environment for the initiation of carcinogenesis in the skin was first described more than 40 years ago. Van der Leun and his colleagues contributed to this area in the 1980s and 90s by experiments in mice involving UV wavelength and dose-dependency in the formation of such tumours, in addition to illustrating both the local and systemic effect of the UVR on the immune system. Since these early days, many aspects of the complex pathways of UV-induced immunosuppression have been studied and are outlined in this review. Although most experimental work has involved mice, it is clear that UVR also causes reduced immune responses in humans. Evidence showing the importance of the immune system in determining the risk of human skin cancers is explained, and details of how UVR exposure can down-regulate immunity in the formation and progression of such tumours reviewed. With increasing knowledge of these links and the mechanisms of UVR-induced immunosuppression, novel approaches to enhance immunity to skin tumour antigens in humans are becoming apparent which, hopefully, will reduce the burden of UVR-induced skin cancers in the future.

Received 18th August 2017,
Accepted 8th November 2017

DOI: 10.1039/c7pp00312a

rsc.li/pps

Introduction

It was first recognised by Kripke and Fisher in 1976 that suppression of immunity followed exposure of the skin to ultraviolet radiation (UVR).[1] They demonstrated that mice, which had been chronically irradiated over a period of time, were unable to reject a highly antigenic UVB-induced tumour on cutaneous implantation (Fig. 1).[1,2] This observation initiated the scientific topic – "photoimmunology". Since that time, many advances have been made to try to understand how exposure of the skin to UVR can have such a profound effect on immunity, not only at the local site of irradiation but also at distant non-irradiated sites. UVR reaching the earth's

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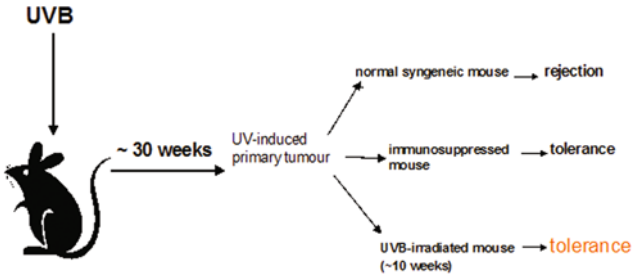

Fig. 1 An illustration of the initial findings of Fisher and Kripke in 1976[1] and 1978[2] which indicated that UVB irradiation of mice led to immunosuppression so that highly antigenic tumour cells were not rejected on transplantation.

surface comprises typically approximately 94% UVA (315–400 nm) and UVB (290–400 nm); UVC (<290 nm) is filtered out by the earth's atmosphere.

The present article is one of sixteen in this issue of Photochemical and Photobiological Sciences commemorating the memory and achievements of Professor van der Leun. Therefore, it is appropriate that our review should start with a summary of van der Leun's contribution to photoimmunology, spanning his publications in the years 1982–1996. The following section describes the diverse and complex pathways leading to local and systemic immunomodulation following UVR exposure. The next section concentrates on the involvement of the immune system in UVR-induced carcinogenesis of the skin. Such tumours are the commonest in fair-skinned populations. They are divided into the non-melanoma skin cancers (NMSCs), basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), and the less frequent but more aggressive melanomas. The final section is more speculative, covering possible strategies for the treatment of skin cancers which rely mainly on interference with the pathways of UVR-induced immunosuppression.

The early days of photoimmunology, illustrated by the observations of van der Leun and his colleagues

The PhD thesis and first papers of van der Leun in the 1960s concentrated on events in the skin surrounding sunburn and blistering. As a physicist working on the cutaneous effects of UVR, he designed artificial UV sources and used mathematical equations to explain the results and to make predictions. It was already known that the UVB waveband in sunlight (290–320 nm) constituted the major environmental risk factor for skin cancer. In the late 1970s, van der Leun and de Gruijl recognised the potential impact of the depletion of the ozone layer on the incidence of skin cancer.[3] Only about 6% of solar UVB reaches the surface of the earth as most is absorbed by the ozone layer. They developed models to estimate the excess number of skin cancers in the human population that would occur with varying degrees of ozone depletion. For example, a 1% decrease in total column atmospheric ozone would result in a 2.7% increase in NMSC.[4] Van der Leun with colleagues used several broadband UV sources to obtain information about the spectral differences in the tumourigenic response.[5] This led to a much bigger study comprising 14 different broadband sources and about 1100 mice which enabled the construction of a more accurate action spectrum (SCUP) for the induction of skin cancer in albino hairless mice.[6] This showed a maximum effectiveness at 293 nm and a small shoulder above 340 nm. Further experiments in hairless mice found that carcinogenicity in the UVA waveband had maximum effectiveness at 365 nm and was 0.9×10^{-4} compared with 293 nm.[7]

In addition to these experiments, van der Leun *et al.* irradiated albino hairless nice daily with a range of UVB doses and assessed the time at which 50% of the animals in a group developed skin tumours, the size of such tumours and their rate of growth.[8] They concluded that the initiation stage was dose-dependent but the growth rate was dose-independent.[8] Furthermore UVB pre-irradiation of the ventral surface of the mice, followed by chronic UVB irradiation of the dorsal surface led to the formation of tumours sooner compared with animals irradiated on the dorsal surface only (Table 1).[9,10] This indicated a systemic effect of the pre-irradiation affecting the initiation stage of tumourigenesis. These results were extended in a subsequent study demonstrating that, if the pre-irradiation consisted of UVA rather than UVB, only a weak systemic effect was apparent.[11] Furthermore van der Leun's group showed that extending the time during which the mice were exposed daily to the same dose of UVB radiation resulted in skin tumours developing more rapidly.[12] In brief, the median tumour induction time was reduced by 12% in mice exposed for 4 or 12 hours per day compared with 1.25 hours per day. Thus the dose rate of UVR is an important parameter in determining its carcinogenic efficacy.

A mouse model was created which demonstrated that UV irradiation prior to sensitisation with the contact sensitiser, picrylchloride, resulted in suppression of the contact hypersensitivity response (CHS) on challenge with picrylchloride.[13] Transfer of lymphoid cells from the spleen and lymph nodes of sensitised donor mice induced CHS in naïve mice after challenge. In contrast, if the donors had been UV irradiated prior to sensitisation, the ability of the lymphoid cells to induce

Table 1 Mean time (days) to the development of skin tumours of different diameters in albino hairless mice following pre-irradiation of the ventral surface in one group and then irradiation of the dorsal surface with FS-40 broad-band UVB sunlamps in both groups. Data from de Gruijl and van der Leun[9]

	<1 mm	1 mm	2 mm
No UV pre-exposure, then daily dorsal UV exposure	74	87	112
UV pre-exposure on ventral surface for 15 weeks, then daily dorsal UV exposure	56	63	82

CHS in naïve mice was considerably reduced. This showed that picrylchloride-specific cells, termed T suppressor cells at the time, were produced in the irradiated sensitised mice that were capable of suppressing CHS. Interest then moved to the possible role of Langerhans cells (LCs) in mediating this UV-induced immunomodulation. Sontag *et al.* showed that LCs containing cyclobutane pyrimidine dimers (CPDs), the most common change in DNA induced specifically by UVR, were present in the lymph nodes draining the site of UVR exposure within one hour of the irradiation.[14] Thus proof was obtained that LCs migrate from the skin to the lymph nodes in response to UVR.

The kinetics of UV-induced suppression as it relates to skin cancer induction was investigated using a highly antigenic skin carcinoma cell line implanted into the ventral skin of hairless mice after they had been subjected to various periods of daily dorsal UV exposure.[15] While the implants failed to grow in unirradiated control mice, they grew in the irradiated mice with the number increasing with UV treatment time and dose. From these and other experiments, it was concluded that tumour induction is dependent on both cumulative UV dose and on time; tumour acceptance is simpler, depending solely on cumulative UV dose. Further study in the hairless mouse model indicated that T cells with suppressor function were already apparent in the spleen and skin draining lymph nodes in the first 1–2 weeks of daily UV exposures leading to carcinogenesis.[16] Thus a bias towards down-regulated immune responses occurred long before foci of UV-transformed cells had formed in the irradiated skin.

Pathways leading to UV-induced local and systemic immunosuppression

As it can take several months to measure the downstream effects of changes in immune cells on tumour growth, the pathways detailed for UVR-induced immunosuppression reflect analyses of isolated cells, cultured skin explants and assays of CHS and delayed type hypersensitivity (DTH) in wild-type and genetically-manipulated mice. Furthermore, as detailed below, pathways of local immunosuppression (reduced responses when antigens are applied to UV-irradiated skin) vary from those of systemic immunosuppression (reduced responses to the antigens used for challenge at body sites distant to the site of UV irradiation and sensitisation). UVR-induced pathways of immune change may be cumulative or redundant depending on the biology and the genes of an individual, and the wavelengths and intensity of UVR to which they are exposed. Different skin types may require different doses of UVR to achieve similar outcomes. The major steps in the pathway leading to suppression of immunity induced by UVR exposure are shown in Fig. 2 and are explained in more detail below.

Where it begins: chromophores initiating UV-induced local and systemic immunosuppression

As UVB wavelengths penetrate into the epidermis but only minimally into the dermis, the energy of UVB photons must be absorbed by epidermal chromophores. As shown in Table 2, these include *trans*-urocanic acid (*trans*-UCA) in the stratum corneum, and components of keratinocytes (DNA, membrane lipids, 7-dehydrocholesterol and tryptophan). As UVA wavelengths can penetrate into the dermis and are poorly absorbed by DNA, they are thought to be absorbed by unidentified cellular chromophores that act as photosensitisers. UVA wavelengths have a greater ability than UVB to generate reactive oxygen and nitrogen species in skin cells, causing 8-oxo-deoxyguanosine (8-oxo-dG) lesions in their DNA.[30]

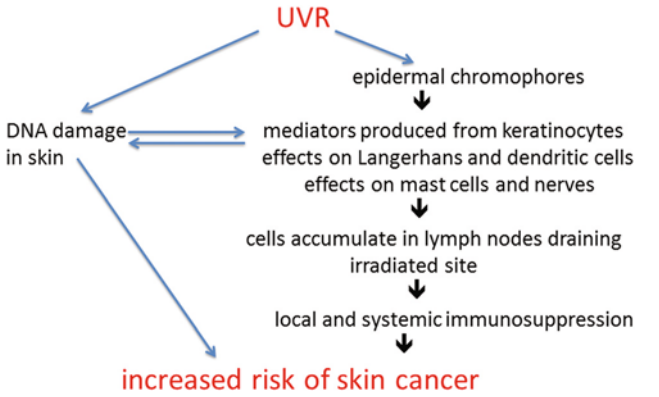

Fig. 2 An outline of the major steps in the pathway leading to the increased risk of skin cancer by the immunosuppression induced by UVR exposure.

Cells and their molecules involved in UV-induced local and systemic immunosuppression

Keratinocytes. The responses by keratinocytes to UVR exposure initiate many of the pathways of UVR-induced immunosuppression. UVB induces the formation of CPDs and pyrimidine (6–4) pyrimidone photoproducts which have been implicated not only in skin cancer development but also in stimulating immune suppression.[22] The membrane lipids of keratinocytes are oxidised by UVR exposure, with a well reported example being formation of platelet activating factor (PAF) and PAF-like lipids.[31] The downstream effects of PAF snowball as keratinocytes express PAF receptors which in turn, make further PAF. Both PAF[24] and *cis*-UCA, the isomer of *trans*-UCA induced by UVR,[18] as well as recognition of UV-induced CPDs, stimulate the production of inflammatory mediators including cytokines (TNF-α, IL-6, IL-8, IL-10, IL-33), chemokines (CCL20), surface markers (RANK-Ligand which is important for signalling DC migration)[32] and cyclooxygenase-2 (COX-2), an enzyme responsible for prostaglandin E_2 (PGE_2) production. Similarly, stress response genes are stimulated in keratinocytes by UVB-activated cytosolic tryptophan when it binds to the cytosolic aryl hydrocarbon receptor (AhR).[26] In recent years, it has been recognised that an increased bioenergetic state is induced in keratinocytes by UVR exposure which permits the inflammatory responses described above. However, if the intracellular stores of nicotinamide and NAD+ are high, they can buffer these inflammatory changes, ultimately reducing the extent of

Table 2 Chromophores initiating UV-induced local and systemic immunosuppression

UVB chromophores		
Chromophore	Immediate products	Role in UVR-induced immunosuppression
trans-Urocanic acid	*cis*-Urocanic acid	Stimulation of keratinocyte inflammatory mediator production and intracellular reactive oxygen species,[17,18] regulation of Langerhans cell,[19] mast cell,[20] and sensory c-fiber[21] activity in UV-irradiated skin.
DNA	Cyclobutane pyrimidine dimers and pyrimidine (6–4) pyrimidone photoproducts	Direct link; immunosuppression reduced upon DNA repair of mutations.[22,23]
Membrane lipids	Oxidation with formation of PAF and PAF-like molecules	Activation of keratinocytes for inflammatory mediator production, and Langerhans cells and mast cells for migration.[24,25]
Tryptophan	Ligand for the aryl hydrocarbon receptor	Activation of keratinocytes,[26] stimulation of dendritic cells to induce T regulatory cells.[27]
7-Dehydro-cholesterol	Vitamin D	Reduction of dendritic cell antigen presenting functions, stimulation of the production and function of T regulatory cells and general dampening of immune responses,[28,29] enhanced repair of DNA lesions.[23]
UVA chromophores		
Unknown	Photosensitisers for increased DNA oxidative lesions	Stimulation of many of the pathways described for UVB chromophores.[30]

UV-induced suppression of immunity and skin cancer initiation.[33,34] Studies with genetically-manipulated mice have further confirmed that production of inflammatory mediators by keratinocytes are mechanistically important to, and not just associated with, UVR-induced immunosuppression.[24] Thus, stimulation of innate immunity by UVR is paradoxically important in UVR-induced immunosuppression and may help to explain non-antigen specific, as well as antigen-specific, suppressed immunity following irradiation of skin.

Absorption of UVB by 7-dehydrocholersterol present in keratinocytes initiates the pathway of vitamin D production; the active form of vitamin D ($1,25(OH)_2D_3$) is homeostatic, and can contribute to immunosuppression. UVR-exposed keratinocytes can be responsible for up to 80% of the body's vitamin D production. In experimental systems, vitamin D can reduce the immunogenic function of dendritic cells (DCs), stimulate the production and function of T regulatory cells (Tregs) and generally dampen immune responses.[28,29] $1,25(OH)_2D_3$ can also reduce the number of both CPD and 8-oxo-dG lesions in human skin, and thus limit UVR-induced immunosuppression.[23] Of interest, both Gorman *et al.*[35] and Schwarz *et al.*[36] have shown that UVR-induced systemic immunosuppression of CHS occurs in the absence of a functional vitamin D pathway.

Langerhans cells (LCs) and dendritic cells. Although once thought to be potent antigen presenting cells, LCs can also have a homeostatic role and migrate to lymph nodes and downregulate skin immune responses.[37] Recent findings suggest that the properties of LCs are determined by the antigen, the extent of barrier disruption and the level of skin inflammation.[37] Migration of LCs from the skin to their draining lymph nodes can be an important event in UVR-induced immunosuppression, particularly for antigen-specific reduced immunity when the skin barrier remains intact.[37,38] It has been proposed that the inflammatory cytokines mentioned above, as well as PAF- and RANKL-expression by UV-irradiated keratinocytes, signal the migration of LCs to draining nodes where they present antigens with altered efficiency, culminating in increased production and function of Tregs.[38] UCA may also regulate LCs,[19] and LCs been reported to activate immunoregulatory natural killer T cells in UV-irradiated skin.[39] UVR exposure also stimulates dermal DCs to migrate to draining nodes. Stimulation of the AhR in DCs reduces their immunogenic potential with the induction of Tregs.[27] To enforce the regulatory environment and in a feedback loop, UVR-induced Tregs can switch DCs from a stimulatory to a regulatory phenotype.[40]

Mast cells. Dermal mast cells are critical players in the ability of UVR[41] and *cis*-UCA[20] to stimulate a systemically suppressed state. This was first shown in mast cell depleted mice in which UVR was unable to systemically suppress CHS unless the irradiated skin had been reconstituted with mast cells.[41] Further, the numbers of dermal mast cells in different strains of mice determine the dose of UVR required for suppression of CHS. Exposure to UVR, *via* production of the inflammatory mediator PAF, increases expression of CXCR4 on dermal mast cells by histone acetylation.[25,42] These cells then respond by migration towards increased expression of CXCL12, the ligand for CXCR4 on B cells in the draining lymph nodes, leading to the production of regulatory B cells in these lymph nodes and suppressed immune responses.[43] Another UVA- and UVB-induced pathway implicated in stimulation of mast cell migration and downstream immunosuppression is the alternative complement pathway and the activity of factor B.[44] Mast cells also respond to $1,25(OH)_2D_3$ by increased production of immunoregulatory IL-10 and therefore increased UVR-induced immune suppression.[45] However mast cells are not important in UVR-induced suppression of local immunity as demonstrated in mast-cell depleted mice when hapten was applied to the UV-irradiated site and reduced CHS still occurred after challenge.[41] This finding has been supported by the evidence that PAF, the molecule described above which stimulates CXCR4 expression and subsequent mast cell migration, does not mediate UVR-induced local immunosuppression.[25]

Nerves and neuropeptides. Skin is an innervated tissue with significant release of neuropeptides upon UVR exposure. In a skin blister model, *cis*-UCA stimulated neuropeptide release from sensory c-fibers and increased blood flow. *cis*-UCA-induced neuropeptides such as CGRP and substance P can also efficiently degranulate mast cells,[21] and/or regulate antigen presentation by LCs.[46] Neuropeptide release from keratinocytes has been implicated in UV-induced immunosuppression; neither UVR nor *cis*-UCA is immunosuppressive in capsaicin-treated, neuropeptide-depleted mice.[47] The UVR-induced neuropeptide, α-melanocyte stimulating hormone, can induce tolerogenic DCs which expand Tregs.[48] UVB-activation of neuroendocrine pathways in skin have also been linked with systemic immunosuppression in mice.[49]

Cells accumulating in the lymph nodes draining UV-irradiated skin. For a greater understanding of UVR-induced immunosuppression, analyses of the number, function and structural positioning of cells in the skin-draining lymph nodes have been important. Apart from resident cells, lymph node cells have migrated from UV-irradiated skin, or have been attracted by chemokines from the circulation. In systemic immunosuppression, there will also be cells from the new site of antigen administration although there is no evidence that DCs from non-UV-irradiated distant skin sites can directly, or by production of soluble mediators (IL-12, PGE_2), affect cellular responses in the nodes of UVB-irradiated mice.[50] After UV-irradiation of skin and application of antigen to the same or distant skin, fewer antigen-specific effector and memory T cells generated, and these cells migrate inefficiently to distant tissues, for example to skin[51] and respiratory tissues.[52] Increased numbers and function of T and B regulatory cells have been reported in draining nodes, due at least in part to altered function of LCs, mast cells and B cells. Recent work also suggests that UV irradiation of skin can alter myeloid progenitor cells in bone marrow. DCs developing from these progenitors have reduced migration and priming abilities,[53,54] and these changes may help to explain some of the long lasting systemic effects following UV irradiation of skin.

The role of the immune system in UV-induced cutaneous carcinogenesis

Evidence that the immune system is important in determining the risk of skin cancer

Recognition more than 30 years ago that the incidence of skin cancer is greatly increased in patients receiving immunosuppressive drugs to prevent rejection of a transplanted organ indicates a major role for immune cells in the control of cutaneous carcinogenesis.

Various surveys in countries round the world have found that the prevalence of SCC is 65–250 times higher, BCC 10 times higher and melanoma 7 times higher in immunosuppressed patients compared with the general populations. Table 3 shows illustrative data for renal allograft recipients. The ratio of approximately 25% SCCs to 75% BCCs found in immunocompetent people is reversed in such individuals and almost all their tumours arise on sun-exposed body sites. In addition, the progression of their tumours is more aggressive than in imunocompetent people, showing a higher than normal metastatic potential and the development of multiple skin lesions. Different types of immunosuppressive drugs are used to prevent rejection of the allografts, some of which such as azathiopurine may increase the risk of skin cancer, not only by reducing anti-cancer immunity but also by their disruptive effects on DNA.[59] Skin adjacent to SCC in allograft recipients has a Th2 expression pattern compared with that in immunocompetent subjects.[60] In addition, the SCCs in allograft recipients contain a higher proportion of $Foxp3^+$ Tregs to $CD8^+$ T cells (perhaps permitting tumour progression) and a higher percentage of IL-22 producing $CD8^+$ T cells.[61]

In addition to allograft recipients, patients with non-Hodgkin lymphoma, including chronic lymphocytic leukaemia, have an increased risk of secondary tumours, with skin cancers (melanoma, SCC and BCC) being most common.[62] Such lesions are aggressive with higher recurrence rates and increased metastatic potential compared with healthy people. Thus, again, the importance of the immune system in controlling skin cancers is illustrated.

Further evidence is provided by individuals who have the rare genetic disease, xeroderma pigmentosum (XP), in which there is extreme photosensitivity caused by an inability to repair DNA following UVR exposure. The incidence of skin cancer is up to 5000 times that of the general population, with SCC and BCC being most frequent although the risk of melanoma is also increased.[63] The tumours develop typically at an unusually early age, often in the first or second decade of life, and predominantly on sun-exposed body sites. Such patients have immune abnormalities which are likely to contribute to the increased risk of cutaneous carcinogenesis. These include a decrease in the relative proportion of circulating T lymphocytes, a reduction in natural killer (NK) cell activity,[64,65] impaired development of contact allergy,[66,67] and decreased production of IFN-γ.[68]

Table 3 The percentage of renal allograft recipients developing skin cancers in the years post-transplantation

Location and reference	Number of patients; study period	5 years	10 years	20 years	30 years
Edinburgh, Scotland[55]	202; 1965–86	1.5		13	
Leiden, the Netherlands[56]	764; 1966–88		10	40	
Queensland, Australia[57]	1098; 1969–94	25	45	70	80
Christchurch, New Zealand[58]	384; 1972–2007	10	22	52	72

A different approach was taken by Kelly *et al.* who measured CHS following exposure to various doses of UVR in people with fair skin (phototype I/II) compared to those with more pigmented skin (phototype III/IV).[69] The former group is at considerably higher risk of developing skin cancer than the latter group. It was found that UV-induced suppression of CHS occurred at lower UV doses in those with phototype I/II than in those with phototype III/IV: the difference was particularly apparent at suberythemal doses.

A further demonstration of differences in innate immune parameters being important in determining skin cancer risk in humans was revealed by assessing mast cell numbers in the skin. It was known that a high density of mast cells in skin tumours is associated with a poor prognosis. Grimbaldeston *et al.* found a correlation between the density of dermal mast cells and susceptibility to skin cancer: the higher the number, the higher the risk of BCC and melanoma.[70] The important role of dermal mast cells in UV-induced immunosuppression was detailed in the previous section.

Two interesting epidemiological studies involving individuals who take non-steroidal anti-inflammatory drugs (NSAIDs) concluded that their use over a period of time decreased the risk of SCC development.[71,72] NSAIDS inhibit the production of COX-2 and PG metabolites which are activated as a result of UVR exposure, thus indicating that these compounds play an importance role in both SCC promotion and immune suppression.

Evidence that UVR exposure modulates the immune system to increase the risk of skin cancer

While there is very strong evidence to regard sun exposure as the major risk factor for skin cancer, details of the immunological changes induced by the irradiation during the induction and progression of such tumours are complex and have not been fully elucidated. Most of the information that is available to date has been obtained in mouse models and it is not certain that exactly the same circumstances occur in human subjects. It is likely that the main effect of the UVR is to induce an inflammatory response, even at suberythemal doses, which, on top of mutagenesis, is sufficient to increase the risk of cutaneous tumours. The inflammation will lead to increased blood flow and vascular permeability, and is thought to be important at all stages of tumour development: initiation, promotion and progression. The details of the pathways involved in both local and systemic immunomodulation that follows irradiation are outlined in the preceding section, and below can be found an outline of the changes which relate specifically to BCC, SCC and melanoma.

Basal cell carcinoma. A detailed study in 2007 of BCCs compared with normal skin revealed immature myeloid DCs associated with the tumours and an increased expression of immunoregulatory cytokines such as IL-4 and IL-10.[73] Tregs ($CD4^+CD25^+Foxp3^+$) surrounded the BCCs as well as being present within the tumours. More recently Omland *et al.* found that $Foxp3^+$ Tregs were abundant in BCCs and in peritumoral skin and, indeed, comprised nearly half of the $CD4^+$ cells around the lesions.[74] A high expression of various chemokines, known to be involved in the attraction of Tregs to cancers, was also found. The presence of Tregs round the tumours may be an important immunological response to chronic sun exposure, allowing the BCCs to develop on mutagenesis.

Squamous cell carcinoma. Data from experiments in mice have provided robust evidence that UV-induced immunosuppression is a major factor in the development of SCCs. Perhaps this has been shown most clearly by the inhibition in photocarcinogenesis if particular steps in the pathway leading from absorption of photons by epidermal chromophores to the generation of particular subsets of cells in the lymph nodes draining the irradiated site. Thus, treatment of mice with a *cis*-urocanic acid monoclonal antibody,[75] a PAF receptor antagonist,[76] or a serotonin receptor antagonist[76] during chronic UVR exposure significantly reduced the number of SCCs that developed. Similarly, inhibition of the production of COX-2, a factor which is overexpressed in chronically irradiated mouse skin, inhibits the formation of UV-induced SCCs.[77,78]

Further information about the contribution of different aspects of the immune system to photocarcinogenesis has been shown in knockout mice. Thus Maeda *et al.*[79] and Jantschitsch *et al.*[80] using mice lacking IL-12 or IL-23 or a subunit of these cytokines found that each play a role in protection against photocarcinogenesis with the loss of both at the same time having the greatest effect. IL-12 and IL-23 are produced in the skin following UVR exposure and they reduce the DNA damage that follows the irradiation. By using chronically irradiated $CD4^{-/-}$ and $CD8^{-/-}$ mice, Nasti *et al.*[81] demonstrated that the number of SCCs and tumour volume were less in the $CD4^{-/-}$ mice than in wild type mice, while a higher number of SCCs occurred in the $CD8^{-/-}$ mice. Also, $CD4^+$ T cells from the skin tumours produced IL-4, IL-10 and IL-17, whereas $CD8^+$ cells produced IFN-γ. Therefore $CD4^+$ T cells promote the development of SCC, with $CD8^+$ T cells being protective. This conclusion was confirmed using mice deficient in IFN-γ in which there was an accelerated incidence of SCC following chronic UV irradiation, indicating a role for IFN-γ dependent type 1 immunity in protection against photocarcinogenesis.[82]

In humans, biopsies of SCCs, ranging from well to poorly differentiated, showed that intratumoral infiltration of Tregs ($Foxp3^+CD25^+$), high expression of TGF-β and IL-10, few plasmacytoid DCs ($CD123^+$) and a low $CD8^+$: $Foxp3^+CD25^+$ ratio contributed to the aggressiveness of the tumours.[83] A further study in human subjects found that the Tregs from SCCs were capable of suppressing effector T cells responses.[84] Such cells were more common in primary SCCs that metastasised than in those that had not. Therefore, a major role for Tregs, potentially induced by UVR exposure, in SCC development and progression is indicated.

Cutaneous human papillomavirus (HPV) types belonging to the beta genus were first detected in the skin of allograft recipients and then of healthy people. Many epidemiological studies have shown that those with SCC, compared with the

general population, are more frequently positive for viral DNA in the skin, or for serum antibodies against the major viral capsid protein. Thus, beta HPV positivity is associated with an increased risk of SCC.[85] HPV DNA is not found in all cancer cells and the viral load is higher in premalignant lesions than in SCC, suggesting that an early stage in the carcinogenic process may be affected by the infection: any interaction of HPV proteins with the immune system following UVR exposure is unknown at present.

Melanoma. Investigating immune responses in melanoma and how they are modulated by UVR exposure are extraordinarily complicated for several reasons. First, there are thought to be two major pathways leading to melanoma: one is found in people with naevi where the tumour is initiated by childhood sun exposure and promoted by intermittent sun exposure thereafter, while the other is found in sun-sensitive people where chronic sun exposure is the key risk factor.[86] Indeed in more than 80% of cases of melanoma, there is no sign of a pre-existing naevus. Secondly, there are different types of melanoma classified according to growth patterns as superficial spreading, lentigo maligna or nodular, by anatomical site (cutaneous, mucosal, acral) and pigmentation, or by the major driver oncogenes. Almost all the mouse models of melanomagenesis involve transplantable syngeneic melanoma lines or are transgenic with enforced expression of mutant oncogenes. Their ability to mimic the melanomagenesis process in humans remains uncertain although they have provided important information linking UVR, immunodulation and melanoma.

Zaidi *et al.* used a transgenic mouse model (hepatocyte growth factor/scatter factor) which develops lesions in stages similar to human melanomas.[87] When these mice were UVB irradiated as neonates, macrophages were recruited to the exposed skin which then produced IFN-γ and this, in turn, promoted the formation and survival of the tumours. UVA did not have this effect. Furthermore, if macrophages were depleted when the neonatal mice were irradiated, proliferation of the melanocytes was reduced.[88] In 70% of melanomas in humans, abundant macrophages producing IFN-γ are present.[87] A recent study found that melanomas release molecules which recruit macrophages and that, with progression, there is a polarisation towards a M2 type of macrophage with release of immunosuppressive cytokines such as IL-10.[89]

A different mouse model was developed by Nasti *et al.* which involved treatment with topical dimethylbenz(a)anthracene and 12-0-tetradecanoyl-phorbol-13-acetate (TPA) followed by TPA twice weekly, thus inducing the formation of pigmented naevi which then become invasive and metastasise to regional lymph nodes.[90] By using such mice knocked out for IL-12 or IL-23, it was possible to distinguish the role of each of these cytokines in melanomagenesis.[91] The IL-12$^{-/-}$ mice developed fewer melanocytic tumours than the wild type mice, while the IL-23$^{-/-}$ developed numerous naevi which progressed rapidly with the likelihood of metastasis. It was concluded that IL-12 supported naevus development with probable metastases, but IL-23 was involved in melanocyte homeostasis, thus inhibiting melanoma development. It did this by augmenting DNA repair induced by UVR, and by limiting Tregs, inhibiting IL-10 production and blocking IFN-γ production. Very importantly, IL-23 also inhibited angiogenesis in the melanoma microenvironment, reduced migration of pigmented cells to the lymph nodes and decreased the infiltration of macrophages which, as described above, favour tumour growth.

With regard to the spread of melanoma, Bald *et al.* repeatedly UV-irradiated primary melanomas in a genetically engineered mouse model.[92] Tumour cells were found to expand along blood vessel surfaces. This was due to the release of the chromatin protein, HMGB1, driven by the toll-like receptor 4 on UV-damaged keratinocytes, together with the accumulation of melanocytes in the upper dermis. Subsequently these changes led to the recruitment and activation of neutrophils which then stimulated angiogenesis and promoted the movement of melanoma cells towards endothelial cells.

Finally, two recent reviews have concluded that sun exposure is probably not a risk factor for melanomas occurring on acral sites (palm, sole of foot, subungual regions).[93,94] In brief, there is no latitude gradient in incidence, no sunburning history in the majority of cases, the tumours have few UV-related mutations and 90% of cases lack a precursor naevus. While trauma or chronic inflammation are likely precipitating factors, the possibility of immunosuppressive mediators resulting from UVR exposure which then down-regulate responses at distant unexposed body sites cannot be discounted.

The focus ahead for treatment of skin cancers: potential of molecules that interfere with, or are independent of, pathways of UVR-induced immunosuppression

UVR-induced lesions in DNA are implicated in both skin cancer development and stimulating immune suppression. Thus, reagents that regulate the repair of DNA and restore immunocompetence are required; in mice, these include 1,25 $(OH)_2D_3$, liposomes containing DNA repair enzymes and antagonists of receptors for PAF and *cis*-UCA.[23–25] Furthermore, in carcinogenesis experiments in mice, 1,25 $(OH)_2D_3$ and nongenomic vitamin D analogues,[95] PAF receptor antagonists,[76] and a CXCR4 antagonist[43] were all successful in reducing the number of UVR-induced skin cancers. Translation of these findings, using mediator-containing, topically applied creams, to reduce the incidence of skin cancer in humans is urgently required. One success has been oral supplementation with nicotinamide shown to reduce the number of new BCCs, SCCs and actinic keratoses, the precursor lesions to SCCs, in immunocompetent individuals by reversal of UVR-suppressed energy-generating processes in skin cells.[34] Another study showed a trend for a similar effect of oral nicotinamide in immunosuppressed patients.[96] Cytokine

receptor agonists may be used in the future; recently it was reported that IL-23 can inhibit melanoma development by augmenting DNA repair and limiting Tregs in the skin.[91] Similarly, agonists of the OX40 co-stimulatory molecule may be able to reverse the suppressive effects of Tregs in SCCs.[97] Inhibitors of mast cell activation, migration and function may also provide a novel anti-skin cancer therapy.

Another pathway of control of UVB-induced immunosuppression and skin cancer formation involves stimulation of endogenous anti-oxidant factors by UVA radiation.[98] Mediators of this pathway, which can be replicated by small molecules, include induction (indirectly *via* oestrogen-receptor-β signalling) of haem oxygenase-1, which in turn catalyses the degradation of haem to biliverdin, carbon monoxide and free iron.[99] For example, carbon monoxide-releasing molecules, as well as oestrogen receptor agonists lead to reduced photocarcinogenesis in mice.[98] This pathway may explain a lower incidence of skin cancers in women. Future research is needed using agonists targeting molecules downstream of the oestrogen receptor for reducing the incidence of skin cancer. It is possible that UVB-induced immunosuppression and the risk of skin cancer formation may also be controlled by exposure, or shielded exposure, to wavelengths of light other than UV. Biological responses have been measured to both blue[100] and red light[101] but their relevance to the control of skin cancer development remains unclear.

A further potentially profitable focus in future may involve metabolomic approaches to investigate as-yet-uncharacterised molecules produced by unirradiated and UV-irradiated skin. In such a discovery project, new molecules identified may be important in homeostasis, inflammatory responses or stimulation of immune suppression and/or skin cancer development. They may indirectly modulate these responses by effects on skin microbiota. Host defence peptides already characterised in skin include LL-37, known as human cathelicidin, which can have pro- or anti-inflammatory properties depending on the cell type and inflammatory stimuli present.[102] UVR-induced vitamin D can enhance LL-37 expression. UV-irradiated skin also produces numerous β-defensins which can stimulate chemokine and cytokine production, and increase keratinocyte proliferation.[102] However, further research is needed to understand the activity of β-defensins in UV-irradiated skin as human β-defensin-1 promotes an immune response to tumour antigens but β-defensin-2 and -3 may stimulate SCC growth.[102]

Stimulation of anti-tumour immunity by mechanisms that do not interfere with UVR-induced immunosuppression may provide a beneficial outcome. Recent interest in the treatment of skin cancers has centred on the use of monoclonal antibodies to block the signalling pathways of programmed death-1 (PD-1) and its ligands, PD-L1 and PD-L2.[103] The former is expressed on lymphocytes, the latter on cancer cells and other host cells including DCs; PD-1 : PD-L1 interaction (described as a host immune checkpoint mechanism) curtails T cell-driven anti-tumour immunity and stimulates subsequent tumour growth. To our knowledge there are no reports that UVR-induced immunosuppression involves PD-1 : PD-L1 interaction. However, in melanoma cells, COX-2 expression correlates with, and positively modulates PD-L1 expression,[104] and COX-2 is induced in UV-irradiated skin.[24] Reduced PD-L1 expression was found in activated DCs differentiated from the bone marrow of UV-irradiated mice,[105] but any consequence of this change is not clear at present. Imiquimod, an immune response modifier acting as a Toll-like receptor 7 (TLR7) agonist, is a therapeutic agent for treatment of BCC. As a powerful inducer of inflammatory responses, imiquimod and similar synthetic ligands of the TLR7 may be used more broadly for treatment of skin and other cancers.[106]

Future anti-cancer therapies may target proliferating skin cancer cells. For example, an antibody to IL-22 has been suggested as an inhibitor of the rapid growth of SCCs in allograft recipients as IL-22 is known to accelerate keratinocyte proliferation, an effect potentiated by cyclosporine which is frequently used as one of the immunosuppressive agents in such patients.[107,108]

Conclusions

This review began with a recollection of the novel and robust findings by van der Leun and his colleagues linking UVR exposures, rates of skin carcinogenesis and immune responses to experimental antigens. Next, the complex pathways of UVB-induced immunosuppression were described, initiated by the epidermal chromophores, followed by the subsequent changes in the irradiated skin and draining lymph nodes. More experimental work is required to determine the nature of UVR-induced immunosuppression, particularly what signals from UV-irradiated skin may be present in distant non-skin-draining lymph nodes and tissues, such as the lung and central nervous system. The following section presented the clear evidence that UVR-induced modulation of the immune system is important in the initiation and development of skin carcinogenesis in humans. The review finished with a consideration of some present and future treatments for human skin cancer, including the links between the immune system and skin carcinogenesis. We propose that this may be a fertile area of research beyond 2017.

Conflicts of interest

There are no conflicts to declare.

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