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# Are there differences in immune responses following delivery of vaccines through acutely or chronically sun-exposed compared with sun-unexposed skin?

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## Summary

**The majority of human vaccines are administered above the deltoid muscle of the arm, a site that is chronically sun-exposed in many people. It is known that exposure of the skin to the UV wavelengths in sunlight stimulates systemic immunosuppression, an outcome that is associated with reduced immunity to microbial infections in animal models. Here we consider whether immunization of humans through a UV-irradiated skin site will lead to a less effective immune response compared with immunization through an unexposed site. Studies showing that the efficacy of vaccination can be reduced when surrogates of increased levels of sun exposure, such as latitude of residence and season of the year, are considered. Results from a limited number of intervention experiments in humans demonstrate a similar pattern. To provide an explanation for these findings, changes in the number and functional potential of immune cells in chronically sun-exposed compared with unexposed skin are outlined. UV radiation-induced changes to skin cells are also relevant when considering skin sites for administration of immune-tolerizing peptides. The review provides the basis for further research into the effects of acute and chronic UV radiation exposure on skin cells in the context of vaccination.**

**Keywords:** dendritic cells; intradermal vaccination; mast cells; memory T-cells; regulatory T-cells; ultraviolet radiation-induced immunosuppression.

## Introduction

The majority of vaccines are administered intramuscularly (IM; Table 1). However, the efficacy of vaccine delivery through intradermal (ID) injection can be greater than that obtained by IM or subcutaneous (SC) injections, and reflects the high concentration of professional antigen-presenting cells in the epidermis and dermis of human skin. Delivery of vaccines ID may thus allow injection of lower doses of vaccine and cheaper large vaccination programmes particularly in developing countries.[1] Recent advances in ID injection technologies such as dissolving microneedles that allow safe self-administration[2] have also helped improve the design and acceptance of ID vaccine programmes. In the era of rapid developments in immunotherapy, ID delivery may enhance the efficacy of an experimental vaccine.

Vaccination through skin utilizes important defence mechanisms that otherwise protect us from external environmental challenges such as allergens, toxins and infectious agents. The skin's robust and finely-tuned immune cell network ensures that the appropriate innate and adaptive immune responses are mounted, possibly including the development of memory cells for a rapid secondary response. The skin immune system also regulates the magnitude and duration of an immune response to

Abbreviations: Breg, B regulatory cell; DC, dendritic cell; DTH, delayed type hypersensitivity; FITC, fluorescein isothiocyanate; HPGD, 15-hydroxyprostaglandin dehydrogenase; ID, intradermal; IL, interleukin; IM, intramuscular; KLH, keyhole limpet haemocyanin; LC, Langerhans cell; $PGE_2$, prostaglandin $E_2$; TCR, T-cell receptor; $T_{FH}$, T-follicular helper cell; Th, T helper cell; Treg, T regulatory cell; $T_{RM}$, tissue-resident memory T-cells; SC, subcutaneous; UVB, ultraviolet B; UVR, ultraviolet radiation

 

**Table 1.** Site of administration of vaccines currently recommended for optimal human health (Australian Immunisation Handbook, Australian Government Department of Health, 23 April, 2019)

| Route of administration | Vaccine |
|---|---|
| IM | Diphtheria-tetanus, diphtheria-tetanus-acellular pertussis, hepatitis A, hepatitis B, *Haemophilus influenzae* type B (Hib), human papillomavirus, inactivated poliovirus combination, Japanese encephalitis (JEspect), 13-valent pneumococcal conjugate, typhoid Vi polysaccharide, meningococcal B, Hib-meningococcal C conjugate, quadrivalent meningococcal conjugate, rabies (chick embryo cells) |
| SC | Inactivated poliovirus, varicella (chickenpox), Japanese encephalitis (Umojev), Q fever, zoster (shingles) |
| IM or SC | Influenza, measles-mumps-rubella, measles-mumps-rubella-varicella, 23-valent pneumococcal polysaccharide, rabies (human diploid cells), yellow fever |
| ID | Bacille Calmette-Guerin |
| Oral | Rotavirus, cholera, typhoid |
| Nasal spray | Influenza |

prevent an over-reaction, cause minimal inflammation and conserve metabolic energy. The fine balance of these processes can, however, be altered, and one of the most common and influential regulators of the rheostat of cutaneous immunity is exposure to ultraviolet radiation (UVR) present in sunlight (for review[3–6]). In mice, if an experimental antigen is delivered to irradiated skin a few days after UVR exposure, the outcome is a dampened or rearranged adaptive immune response to that antigen, with the development of fewer memory cells. In humans, UVR exposure can reduce sensitization to a hapten applied to the irradiated site that is manifest as a reduced inflammatory response to challenge by that hapten at an unirradiated skin site.[7] Furthermore, the magnitude of a response is significantly reduced when a challenge antigen is administered to a recently UV-irradiated site on an antigen-sensitized person.[8]

Thus, it is possible that skin that has been exposed chronically to UVR may not be the optimal site for delivery of vaccines, and unexposed skin should be used instead. However, the effects of UVR exposure on skin immunity may be short-lasting and contribute little to responses to antigens delivered on, or under, chronically sun-exposed skin. Alternatively, if the systemic effects of UVR exposure are sufficiently robust, neither the site of antigen sensitization and/or challenge may be important. In this article, the term 'chronically sun-exposed' refers to the normal day-by-day exposure of skin to sub-erythemal or occasional erythemal doses of solar UVR, such as above the deltoid muscle in the upper arm or the posterior aspect of the forearm. The term 'unexposed' refers to sites such as the buttock and the inside of the upper arm or antero-lateral aspect of the mid-forearm. After a brief introduction to the immunoregulatory pathways stimulated by UV-irradiation of skin, the associations of sun exposure and immune responses in humans to contact allergens, skin infections and ID antigens are presented. This is followed by a description of interventional studies in which UVR is delivered, or UVR exposure reduced, at times close to antigen or vaccine delivery. The next section addresses possible determinants of outcome by considering the number and function of several populations of immune cells after application of antigens through sun-exposed skin compared with unexposed skin. The longevity of the effects of UVR exposure and the chronicity of UVR exposures that may alter immune responses to topical or intracutaneous antigens have not been previously reviewed. It should be noted that the increased melanin content of chronically sun-exposed skin can reduce the synthesis of mediators such as vitamin D,[9] thus decreasing the immunomodulatory effects of UVR and affecting the behaviour of immune cells. In addition, chronic sun exposure may alter the skin microbiome[10,11] and, in turn, ensure homeostasis by changing the number and activity of immune cells in skin. These aspects are not examined further in this review.

The skin sites that allow maximal long-lasting immunity to vaccine antigens are central to this review. It is also relevant to consider the design of strategies for tolerizing immunotherapies using peptides and other antigen formulations that may be delivered SC, ID or transdermally, and that rely on the skin immune system for their efficacy. Administration of such formulations via sun-exposed or unexposed skin may depend on the ability of UVR exposure to alter the function of cells, such as dendritic cells (DCs) and T regulatory cells (Tregs) that govern outcomes in immunotolerizing protocols.

## The skin immune system and UVR-induced immunosuppression

The response of skin resident cells as a result of direct or indirect activation by UVR and their immune functions in the epidermis and dermis have been recently reviewed.[3,5,12] These cells include not only epidermal keratinocytes, but also dermal lymphocytes, nerves and mast cells. Of importance to ID challenge with vaccine antigens, the skin contains many phenotypically distinct DC populations that are versatile and 'plastic' in their antigen-presenting properties. Although some skin DCs may be intrinsically more efficient than others at cross-presentation or activating T-follicular helper cells ($T_{FH}$), the functions of Langerhans cells (LC; for topical antigens) and dermal DCs (for ID antigens) are largely determined by their tissue microenvironment (for review[13]). After decades of research generally using experimental antigens


 

and frequently in mice, the following processes underpin UVR-induced systemic immunosuppression (for review[4,5,12,14]). Cells in UV-irradiated skin produce multiple mediators that modulate skin DCs to regulate T-cell-dependent responses in the skin-draining lymph nodes, including reduced T helper cell (Th)1- and Th17-driven responses, less induction of effector memory cells, and greater production and increased function of Treg and B regulatory (Breg) cells. Signals from UV-irradiated skin may also reduce the induction of $T_{FH}$ cells in lymph nodes,[15] leading to diminished humoral immunity. Peripheral Tregs induced by LC/DC presentation of antigens applied to, or injected into, UV-irradiated skin may, if receiving the appropriate signals and cytokines, proliferate and/or migrate from the lymph nodes into the circulation[16,17] or back to UV-irradiated skin where they can modulate the inflammation associated with skin disease.[18] The phenotype and longevity of UVR-induced regulatory lymphocytes are discussed below. UVR-induced immunomodulatory mediators, such as *cis*-urocanic acid, interleukin (IL)-4, IL-10 and prostaglandin $E_2$ ($PGE_2$), may also extend beyond draining nodes and have been measured in the circulation and urine. At least in mice, UVR-induced regulatory cells and soluble mediators may regulate DC activity in lymph nodes draining unirradiated sites and reduce sensitization to new antigens.[19,20] Tolerance may be an extension of UVR-induced suppressed responses to an antigen, and is the desired outcome of UVB-phototherapy for chronic inflammatory skin conditions in humans. Immunotolerance to antigens driving the development of multiple sclerosis was also sought in a recent trial of narrowband UVB phototherapy for individuals with clinically isolated syndrome, a pre-form of multiple sclerosis.[21]

## Associations suggesting that sun exposure suppresses immune responses to infections and to vaccines

Vaccines to protect against a range of human infections include live-attenuated, inactivated and subunit forms. Particularly in the context of the live-attenuated ones, it is of interest to consider investigations that have monitored changes induced by UVR in the immune responses to infectious agents. Animal models, involving mice, rats and guinea pigs, have shown consistently that UVR exposure prior to (or following in some cases) microbial infection resulted in reduced microbe-specific T-cell immunity, frequently with an increased microbial load, severity of symptoms and even death on occasion. The organisms studied included viruses, bacteria, fungi, protozoa and nematodes (for review[14]). The most recent of these studies involved irradiating mice with a low dose (0·1 minimum erythemal dose) on four consecutive days or a single high dose (two minimum erythemal doses) of broadband UVB before SC infection with *Staphylococcus aureus* on the following day.[22] Suppression of T- and B-cell responses to the bacteria occurred after the high dose but, in contrast, an enhancement of these responses occurred after the low doses. However, the latter effect did not lead to more effective control of the spread of the infection, perhaps due to some aspects of the adaptive immune response not being generated.

In four studies carried out between 1992 and 2009, animals were vaccinated with *Leishmania major* (ID), *Candida albicans* (SC), *Mycobacterium tuberculosis* (ID) and herpes simplex virus (SC), prior to or soon after exposure to UVR, and then challenged at a later date with the live organism. In all cases there was an increased microbial load in the irradiated animals and, where tested, a reduced microbe-specific cell-mediated immune response in the irradiated animals compared with the unirradiated (for review[14]). In the first three of these studies, the immunomodulatory effects were shown to be systemic as challenge with the live organisms took place at sites that were not irradiated around the time of the vaccination.

Although such vaccination models are unnatural and were investigated at a time before reagents for identifying T-cell subsets and many immune mediators were available, they provide an indication that UVR could reduce the efficacy of vaccination in humans. Furthermore, several latent viruses in humans can be reactivated by irradiation. Several examples follow. First, exposure to solar UVR is a common trigger for reactivation of herpes simplex virus to cause ocular[23] or skin lesions.[24] The ability of antigen-presenting cells in the irradiated epidermis to present the viral antigens to autologous T-cells was reduced.[25] Secondly, the incidence of shingles, caused by the reactivation of herpes zoster virus, is higher in the summer than in the winter months in countries as diverse as Poland, South Korea, Taiwan and Australia.[26,27] Thirdly, in HIV-infected men, the risk of Kaposi sarcoma, which is associated with human herpesvirus 8, was increased in those who resided at locations with high ambient UV radiation or who had developed a keratinocyte cancer (as a proxy for lifetime solar UVR exposure) before developing Kaposi sarcoma.[30]

Investigations into the impact of UVR on the efficacy of vaccination in humans are sparse. Those that rely on correlates of sun exposure, such as vaccination in the winter versus the summer and differences in latitude of residence, are outlined in Table 2, while intervention studies are described in the next section. It should be noted that, in some of the reports in Table 2, exposure to solar UVR was not considered by the investigators as a possible contributory factor to account for the results. In addition, the chronology of the vaccination procedure with respect to sun exposure, the personal solar UVR exposure around the time of the vaccination and other potential confounding factors were unknown. However, when assembled together, the data from a wide range of

**Table 2.** Summary of evidence that solar UVR may reduce the efficacy of vaccination in humans (cited in chronological order)

| Vaccine | Location of study | Study population | Effect of UVR | Reference |
|---|---|---|---|---|
| Poliovirus | India | 22 infants plus 169 aged 1−5 years | Antibody response lower than in similar studies in temperate areas | John & Jayabal (1972)[31] |
| Poliovirus | Israel | 226 infants | Antibody response higher if vaccine administered in the winter versus summer | Swartz *et al.* (1972)[32] |
| Influenza virus | Russia | 292 (type A, H3N2) and 296 (type B), aged 16–18 years | Immunogenicity higher if vaccine administered in the winter versus the summer | Zykov & Sosunov (1987)[33] |
| Hepatitis B virus | The Netherlands | 522 students | Initial antibody response higher if vaccine administered in the winter versus the summer | Termorhuizen *et al.* (2002)[34] |
| Measles virus | India | 1103 children | Immunity wanes with high solar UVR exposure | Sharma *et al.* (2004)[35] |
| Measles and polioviruses | Russia | 17 children aged 1–3 years | UVR promotes a Th2 cytokine response | Snopov *et al.* (2005)[36] |
| Rubella virus | Israel | 203 children aged 4–5 years | Antibody response higher if vaccine administered in the winter versus the summer | Linder *et al.* (2011)[37] |
| BCG (tuberculosis) | 10 randomized trials in USA, India, Canada, UK, South Africa, Haiti; latitude 10°–50° | 300−50 000 in each trial vaccinated | Vaccine more protective against tuberculosis with increasing distance from the equator | Mangtani *et al.* (2014)[38] |

vaccines show that UV-induced downregulation in vaccine efficacy is indeed possible, with effects on both humoral and cell-mediated responses.

Recently, a role for vitamin D in this process was suggested. A negative association has been reported between vitamin D status and measles antibody titres in participants in the large National Health and Nutrition Examination Survey in the USA.[39] An involvement of vitamin D was also proposed in a study in which the antibody response to rubella vaccine in children was lower when the vaccine was administered in the summer versus the winter months.[37] Furthermore, higher titres to papillomavirus correlated with lower vitamin D status 1 month after administration of three doses of human papillomavirus vaccine in males, aged 18–25 years.[40] While vitamin D might modulate the immune response to these viral vaccines, it is also possible that an immunosuppressive effect may occur following exposure to UVR (thus increasing vitamin D status) by a pathway not directly involving the production of vitamin D.[3]

## Intervention studies in humans in which recent sun exposure modulates immune responses to sensitizing antigens and to vaccines

Few attempts have been made to monitor real-life sun exposure with the generation of immunity to a vaccine. Although not using a vaccine as such, a suppressed delayed type hypersensitivity (DTH) occurred when seven common microbial antigens were applied to either a sun-exposed or an unexposed skin site following sun bathing for several hours each day for 6 days in the summer in Turkey.[41] In a similar vein, volunteers, vaccinated previously with Bacille Calmette Guerin (attenuated *Mycobacterium bovis*), were irradiated on the lower back with a sub-erythemal dose of solar-simulated radiation on five consecutive days.[8] Purified protein derivative was then injected ID into the irradiated skin site and a distant non-irradiated site and the DTH responses assessed (Mantoux test). Suppression was found at the irradiated site but not at the non-irradiated site. Thus, these two studies indicate that UV-irradiation has the potential to reduce already established T-cell responses to sensitizing antigens and to a vaccine.

There has only been a single clinical trial in which human volunteers were irradiated prior to vaccination with subsequent monitoring of their immune response to the vaccine. About 100 healthcare workers in Utrecht in the winter months were whole-body irradiated with one minimum erythemal dose of solar-simulated radiation, while the same number were unirradiated to act as controls.[42] All were then vaccinated IM with recombinant hepatitis B surface antigen. This preparation contained aluminium hydroxide as an adjuvant. The vaccination was repeated 1 and 6 months later. It was found that the natural killer cell activity and contact hypersensitivity


 

responses were suppressed in the irradiated group compared with the unirradiated group, but no difference between the two groups was found when hepatitis B-specific T-cell activity or antibody responses were assessed. Subsequent analysis of cytokine polymorphisms showed that those subjects in the irradiated group with the minor variant of IL-1β had lower hepatitis B-specific antibody responses.[43] A fourfold increase in IL-1β production results from this variant, which could lead to raised prostaglandin levels and then higher IL-4 and IL-10, thus explaining the reduction in antibody level. Furthermore, volunteers with a higher content of immunoregulatory *cis*-urocanic acid in their skin following the UVR exposure and prior to vaccination had reduced T-cell responses to the vaccine.[44] Thus, while the irradiation did not cause downregulation of immunity to the vaccine in the exposed group as a whole, this study indicated that there may be interpersonal genetic and biochemical differences that may determine whether the immune response is modulated by the UVR. In addition, the vaccine used in this study was administered at a high dose to elicit protective immunity in the majority. A clinical trial using a vaccine not containing an adjuvant, given at a lower dose, and administered ID may allow a more valid assessment about the impact of UVR on the generation of immunity to the vaccine.

Wright *et al.*[45] conducted a sun protection intervention study involving approximately 100 Black African children in a rural setting in Limpopo Province, South Africa (UV Index 10–14 at midday in summer). The primary measles vaccine (live attenuated) was given IM in the thigh or upper arm at 6 months of age and the booster at 18 months. At the time of the booster, the parent or guardian of approximately half of the children was asked to ensure that their child used the sun protection equipment provided (bucket hat, long-sleeved shirt, umbrella to shield the child when carried outdoors, broad-spectrum sunscreen − sun protection factor 30), and was given advice to avoid the sun between 11 am and 2 pm and to seek shade for the following week when their child was outside. The other half of the children acted as a control group. At 4 weeks, all children in both groups achieved levels of measles-specific IgG above those considered to provide protection and no difference between the two groups was found.[46] The practical difficulties associated with this study are recognized to be large, but it would be of value in any further associated investigations to consider sun avoidance for several days prior to the booster, measurement of measles-specific T-cell responses and antibody subsets, administration of the booster vaccine by a route involving the skin, sun avoidance prior to and following the primary measles vaccination, and an increase in the number of children involved.

A recent report describes the effect of personal sun exposure on the immune response to the SC administration of keyhole limpet haemocyanin (KLH), a T-cell-dependent antigen, on the forearm.[47] The exposure was clothing-adjusted and measured in 217 healthy adults for 5 days before and after the immunization. The range was diverse as recruitment occurred throughout the year and the subjects lived in two parts of Australia that had different ambient UVR conditions: temperate in Canberra and tropical in Townsville. Subsequent assessment of the immune response to KLH took place up till 29 days after the vaccination. At this time, the DTH to challenge with KLH on the same body site as the original vaccination was lower in those individuals with higher personal clothing-adjusted UVR exposure on the day of immunization and for 2–3 days after it. In addition, an increase in Th17 cells, as a proportion of $CD4^+$ T-cells in the blood, correlated positively with the quantity of personal clothing-adjusted UVR. In contrast, no impact of UVR on KLH-specific antibody responses, and no associations between the immune response to KLH and lifetime UVR exposure or vitamin D status were found. It was concluded that natural exposure to higher personal doses of solar UVR around the time of primary vaccination can reduce vaccine efficacy.

## Cellular changes in chronically sun-exposed skin that may alter protective immune responses to intradermal vaccination

As reviewed above, UVR exposure(s) occurring around the time of vaccination may downregulate the immune process, and thus reduce vaccine efficacy. However, it is important to consider whether the effects of UVR on immunity to vaccines are long-lasting or not. In addition, photoadaptation (UVR-induced immune changes are reduced or no longer occur following chronic UVR exposures) and photoprotection (no downregulation of immunity induced by a high UVR dose) may develop following chronic UVR exposure. As previously reviewed,[48] irradiation of individuals for 10 consecutive days with 0·7 minimum erythemal dose UVR protected to a limited extent against the effect of an erythemal UVB dose on skin cytokine expression and thymine dimer formation, but not on expression of the cyclo-oxygenases and extent of suppression of a contact hypersensitivity response. In fact, cyclo-oxygenase expression continued to increase for 30 days after multiple UVR exposures. With reference to longevity of UVR effects, whole-body UVB irradiation on each of 30 consecutive days with 1·2 standard erythema doses (approx. 0·3 of a minimum erythema dose) significantly and additively reduced a contact hypersensitivity response to a topical hapten and suggested long-lasting cumulative immunosuppressive effects. Therefore, although the evidence is limited from these and other studies (for review[48]), it is unlikely that either photoprotection or photoadaptation, with respect to UVR-induced immunomodulation, develop.

Multiple cell types in the skin may be altered in number and function by chronic exposure to UVR, and regulate immune responses to primary vaccines and boosters when they are applied to chronically sun-exposed skin. UVR-induced changes to skin DCs, Tregs and mast cells, as well as memory T-cells [central and effector memory cells, tissue-resident memory T-cells ($T_{RM}$)] that may contribute to the outcomes of challenge responses, are now considered and illustrated in Fig. 1.

### Dendritic cells

Dendritic cells do not proliferate in skin. As DCs migrate to lymph nodes upon UVR exposure, skin antigens are taken up by macrophages that are inherently poor antigen-presenting cells and may explain reduced 'local' immunity for several days.[49] The efficiency of DCs subsequently engrafting UV-irradiated murine skin is being investigated currently. There have been several recent reports of heterologous effects of vaccination in humans causing protection against non-targeted pathogens in addition to the vaccine pathogen, and have been associated with vaccine-altered metabolic changes in myeloid cells.[50,51] Similarly, in mice, erythemal and repeated sub-erythemal UV-irradiation of skin induces the development of DCs from the bone marrow with reduced expression of a glycolytic enzyme and consequent reduced ability to migrate to chemotactic signals both *in vitro* and *in vivo*.[52] This finding may explain why there were fewer fluorescein isothiocyanate (FITC)+ cells in the lymph nodes draining the FITC-painted ventral skin of mice that had been UV-irradiated on their dorsal skin compared with unirradiated controls.[20] The antigen-presenting function of FITC+ cells in the ventral lymph nodes was equivalent and, thus, systemic immunosuppression observed after erythemal UVR may reflect, in part, UVR-induced alteration to the metabolism and motility of DCs. No change in numbers of blood DCs or DC subsets was detected in individuals receiving repeated whole-body sub-erythemal UVB irradiation, except for a small increase in the percentage of a myeloid cell subset.[53] Further information regarding the metabolic, migratory and functional capacity of these cells is required. If the changes recorded in the murine DCs are replicated in human DCs, a more sustained effect of UVR exposure on DC function would be confirmed. By inducing the production of 1,25-dihydroxy-vitamin $D_3$ from skin DCs, chronic UVR exposure may increase skin-homing CCR10 expression on responding T-cells[54] and, thus, alter the immune equilibrium in skin. In addition, aging and chronic sun exposure can reduce the number of LCs and their morphology (loss of dendrites) in human skin,

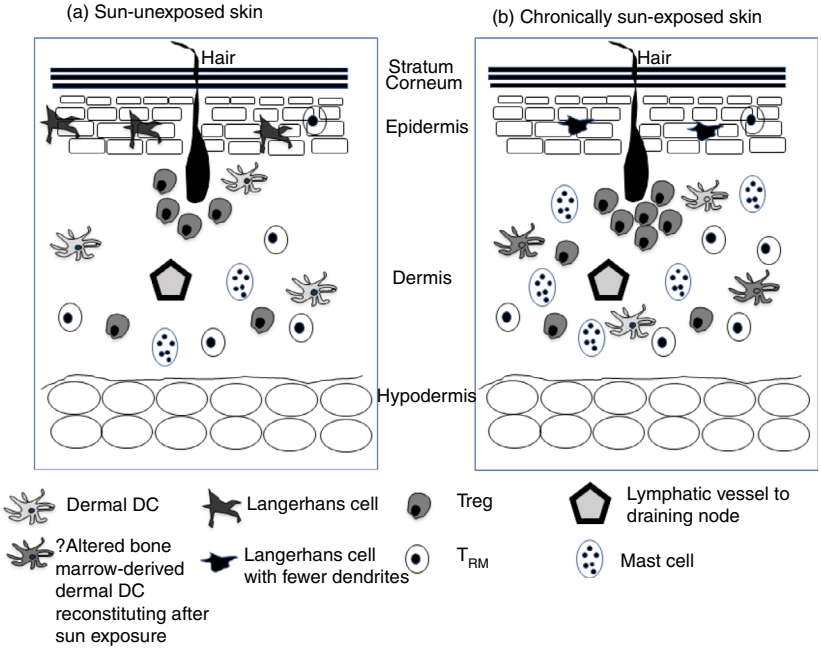


**Figure 1.** Differences in cell populations in (a) sun-unexposed compared with (b) chronically sun-exposed skin. The focus is on changes in dermal cells, although a reduction in aging sun-exposed skin of Langerhans cells (LCs), and their dendrites[55] are shown. Altered numbers of regulatory cells in the epidermis and hypodermis are possible. The numbers of T regulatory cells (Tregs) and mast cells increase in chronically sun-exposed dermal skin. Tissue-resident memory T-cells ($T_{RM}$) may increase particularly if the skin barrier is broken as a result of erythema. Dendritic cells (DCs) in chronically sun-exposed dermal skin may have altered function as the skin may be reconstituted after ultraviolet radiation (UVR) exposure by epigenetically altered cells from the bone marrow.


 

possibly in an additive fashion, and reduce their antigen-presenting ability.[55]

### T regulatory cells

Up to 22 subsets of human Tregs have been identified.[56,57] Circulating Treg subsets have been associated with UVR exposure.[58] In 350 individuals, the proportion of circulating Tregs did not associate with quantitative measures of UVR exposure but the Treg subpopulations with an activation associated phenotype (CD45RA-CD27-), and those expressing skin homing receptors, were significantly and positively associated with UVR, particularly in lighter-skinned individuals.[58] Foxp3 is an essential transcription factor for development of Tregs, and the epigenetic markings on Foxp3 dictate Treg stability.[57] The Foxp3 epigenetic signature of UVR-induced human Tregs has not been studied to date, nor of Tregs induced from T conventional cells *in vitro* by molecules produced in UV-irradiated skin (e.g. *cis*-urocanic acid,[59] nitric oxide[18]).

The ability of Tregs to alter immune responses in skin is governed by their T-cell receptor (TCR) dependence. In normal human skin, Foxp3+ Tregs comprise ~20% of CD4+ T-cells (compared with 5–10% in blood), and murine studies suggest they proliferate under DC direction in response to sub-erythemal UVR (to up to 60% of skin T-cells).[60,61] However, these Foxp3+ cells, as determined by their Foxp3 hypomethylation patterns, originate in the thymus; they may have migrated to the skin in early life and evolved to regulate responses to self-antigens released from UVR-damaged cells. The numbers of Foxp3+ Tregs in the skin are also maintained by keratinocyte IL-7 and processes dependent on resident skin microbes[62] and UVR-induced antimicrobial peptides.[63] Extensive phenotyping of human UVR-induced Treg is required as well as studies of their antigen specificity and mode of action. Tregs produced in response to a contact allergen applied to UV-irradiated murine skin regulate T-cell responses by production of IL-10. Tregs, at least in mice, can therefore have bystander regulatory effects once activated by their cognate antigen (for review[64]). It is not known if UVR-induced Tregs, like some Treg subsets, express TLRs and other pattern recognition receptors that recognize inflammatory mediators and, if activated, secrete immunosuppressive cytokines such as IL-10.[65,66] As a TCR-independent response, some Treg subsets in human skin can mediate tissue repair by producing the growth factor, amphiregulin.[67] A further advance is the recognition that human Foxp3+ Tregs express high levels of the enzyme 15-hydroxyprostaglandin dehydrogenase (HPGD) independently of TCR stimulation.[68] Importantly, human Tregs have enhanced suppressive function in the presence of $PGE_2$. This is dependent on the ability of HPGD to catabolise $PGE_2$ into 15-keto-$PGE_2$, a molecule that inhibits proliferation of T-cells. As UVR can stimulate both $PGE_2$ production and increase numbers of Tregs in skin, a strong immunosuppressive environment should be created. In summary, the phenotype, the specialized function and the longevity of UVR-induced human Tregs in chronically sun-exposed skin remain largely unknown. If the numbers of Tregs in human skin are increased and then maintained by repeated UVR exposures, they may contribute to the arsenal of cells in skin that provide immunological homeostasis.

### Mast cells

Mast cell numbers increase in chronically sun-exposed skin in humans.[69] Further, in murine studies, the numbers of dermal mast cells determine the extent of susceptibility to UVR-induced suppression of contact hypersensitivity responses.[70] With a higher density of mast cells in the skin of C57BL/6 mice than in BALB/c mice, this strain is more sensitive to low doses of UVR for suppression of immune responses. Both UVR-induced 1,25-dihydroxy-vitamin $D_3$[71] and platelet-derived growth factor can stimulate murine mast cells for IL-10 production and thus immunosuppression.[4] As a result, in chronically sun-exposed skin with a higher number of mast cells, a more pronounced immunosuppressive environment may be maintained.

### Memory T-cells

In mice, UVR exposure at the time of antigen sensitization induces fewer T effector cells than normal and therefore fewer T memory cells, which include both central and effector memory cells, as well as $T_{RM}$. $T_{RM}$ are derived from precursors that enter the tissue during the effector phase of an immune response and remain positioned within this compartment for a quick and powerful response after TCR activation.[72] Exposure to UVR can alter the balance in the skin microbiome of mice (for review[10]) and with skin barrier disruption, UVR exposure may have dynamic effects on $T_{RM}$ developing to skin microorganisms. Some inflammatory skin diseases may reflect cycles of stimulation of $T_{RM}$ in skin to commensal organisms. In turn, $T_{RM}$ can sense UVR-induced injury and contribute to DNA repair.[73]

Persistence of $T_{RM}$ in skin can be stimulated by ligands for the aryl hydrocarbon receptor,[74] which are produced in UV-irradiated skin (for review[5,6]). At the present time, it is not possible to predict whether the development, proliferation and maintenance of vaccine antigen-specific $T_{RM}$, and their responses upon vaccine antigen challenge, are influenced by the administration of antigen through unexposed or chronically sun-exposed skin.[72,75] There is no evidence that $T_{RM}$ induced by UVR-damaged skin

cells or commensal microorganisms will cross-react with vaccine antigens. However, if bone marrow-derived DCs repopulating chronically sun-exposed skin process vaccine antigens less efficiently than those in unexposed skin, fewer $T_{RM}$ may be induced.

## Conclusions

There is considerable evidence that sub-erythemal UVR exposures can reduce immune responses to experimental, skin tumour and vaccine antigens in humans, as in rodents. However, the central question of the longevity of this immunomodulation is important, particularly if the UVR exposures and the closeness in time to antigen delivery for immunity or tolerance are not controlled. Seasonal effects of poorer vaccination outcomes in summer suggest that *ad hoc* and unregulated UVR exposure is sufficient for reduced sensitization to immunizing antigens. Similarly, vaccination is less successful at lower latitudes than at higher latitudes. Several cells in chronically sun-exposed murine skin may be altered in number and/or function and govern reduced responses to vaccination, but this requires confirmation in human studies. Immunosuppression following UVR exposure may enhance immunotolerizing protocols. However, in some experimental models, systemic effects are indicated as UVR reduces sensitization to antigens applied to both UV-irradiated as well as non-irradiated sites. Further studies are required to determine the robustness of the systemic effects of UVR exposures in humans, and whether the immunoregulation is similarly potent for immunogenic and immunotolerizing vaccines delivered to chronically sun-exposed compared with unexposed skin. It is unclear at present if the effect of chronic sun exposure is short-lived or if the systemic mediators and cellular changes are long-lasting. The benefits of ID vaccination are many, including use of the skin's robust immune system that enable lower amounts of vaccine to be given. New methodology has enabled safe and reliable administration protocols. Also, adjuvants have been developed for effective responses to ID vaccination[76] and, in some cases, non-adjuvanted vaccines have been tested.[77] Important considerations for future investigations into optimal ID vaccination include the extent of vaccine sparing and the choice of skin site.

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## Disclosures

The authors have no competing interests to declare.

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