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UV exposure and protection against allergic airways disease

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Asthma is a chronic inflammatory disease of the small and large conducting airway mucosa characterised by Th2 cell immunity. Allergen-specific IgE levels control the immediate response whilst the interplay between airway mucosal antigen presenting cells, Th2 effector cells and CD4+CD25+ regulatory T cells control the late phase, cell-mediated response. Using two experimental systems in mice with ovalbumin and papain, respectively, as the allergens, UV irradiation of skin prior to allergen sensitisation reduced the expression of allergic airways disease, particularly the late phase response. In this review, the reduced Th2-driven, asthma-like responses in respiratory tissues of UV-irradiated mice are detailed. Possible mechanisms of UV regulation are debated. The potential beneficial effects of UV irradiation of skin in controlling allergic airways disease are discussed. This review gives some scientific understanding to century-old anecdotal reports that beach and mountain resort holidays associated with increased UV exposure are beneficial in asthma treatment.

Introduction

Erythema, oedema and the initiation of skin neoplasms are well-known consequences of irradiation of skin with UVB (290–315 nm). Skin cancers develop because immune responses that would otherwise destroy UV-damaged cells are reduced following UVB exposure.^{1,2} UVB-induced immunosuppression is systemic such that immune responses to challenges by infectious agents, tumour antigens and autoantigens at other body sites are also reduced.^{1,2} For three decades now, scientists have been seeking to

better understand the mechanisms by which UVB-irradiation is immunomodulatory.

The terminology that UV causes an immunosuppression is used. However, it was suggested in 1989 by Daynes and colleagues that UV does not stimulate a general immune suppression but more a transition of response from one being Th1-driven to one controlled by Th2 cells.³ They reported that in normal mice exposed to UV radiation there is a dramatic change in the pattern of lymphokines secreted by activated T cells. They found that lymphocytes isolated from UV-irradiated mice produced lower levels of IL-2 and IFN- γ when activated *in vitro* with anti-CD3. The secretion of IL-4 by Th2 cells in these lymphocyte cultures was however increased. In the 1990s, this concept that UV irradiation of skin induced a shift from Th1 to Th2 responses was popular. The arguments were presented by Ullrich in a review in 1996.⁴ These reflected the biological activities of IL-10 produced

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of UV-induced vitamin D on the cellular immune responses that control diseases like asthma.

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Jacqueline McGlade graduated with a PhD from the University of Western Australia in 2008. Her PhD studies centred on the control by UV irradiation of skin on two models of allergic airways disease. Central to her investigations were studies of three tissues involved in expression of human allergic asthma, namely the trachea, the lung and bronchoalveolar lavage fluid, and the airway-draining lymph nodes.

by keratinocytes⁵ and infiltrating macrophages in UV-irradiated skin.⁶ IL-10 not only stimulated antigen presenting cells in the irradiated skin to induce impaired Th1-driven responses, but to bias responses to those dependent on IL-4 and IL-10 production. Also, UV irradiation of skin increases serum IL-10 levels which in turn can be responsible for suppression of Th1 activation and enhancement of Th2 responses upon antigen exposure at distant body sites.⁷ These observations were coupled with reports that administration of IL-12 which can promote the activation of Th1 cells can overcome the effects of UV-induced immunosuppression.⁸ However, with time, there was less support for the argument that UV enhanced Th2-type responses. One characteristic readout of Th2-cells, and of IL-4 that Th2 cells produce, is their association with the development of immunity characterised by IgG1 and IgE antibodies. The effects of UVB exposure were analysed on responses to vaccination with hepatitis B.⁹ A Th2-type response to the vaccine was encouraged by completing the study in BALB/c mice (Th2 prone) and vaccination with alum as adjuvant and strong inductant of a Th2 response. Cellular immunity to the vaccine antigen was reduced, as were IgG2a levels. There was also a suggestion of UV-induced suppression of IgG1 levels in UV-irradiated BALB/c mice.⁹ There was no evidence of UV-enhanced Th2-driven responses, a conclusion supported by studies of reduced IgE/IgG1 levels in UV-irradiated, OVA sensitised mice¹⁰ (see below).

Allergic airways disease as a Th2-driven immune response

Asthma is now a major disease of both the developed and developing world. It is estimated that 1 in 3 children in Western countries have childhood asthma.¹¹ In asthma, airway mucosal antigen presenting cells stimulate Th2 cell immunity and disease.^{12–16} The airway mucosal inflammatory response involves an influx of activated Th2 cells and eosinophils, airflow obstruction, and airway hyper-responsiveness. Products of activated T cells following allergen exposure contribute to airway hyper-responsiveness.^{17,18} The responses in the airway mucosa between mucosal antigen

presenting cells and T memory cells are bidirectional; the activated T cells can remain in the mucosal airway whilst the allergen-loaded antigen presenting cells migrate to the draining lymph nodes for allergen presentation to further immune cells, and the prolongation of the asthmatic response.^{12–16} Activated T cells migrate in to and out of the mucosal airway in cyclical waves.¹⁹ Changes in the function of airway mucosal antigen-presenting cells, airway T memory and T regulatory cells determine the outcome of allergen challenge in the airways.

This review covers studies by us and others of the effects of UV irradiation of skin on murine asthma models. These studies do not suggest that UV irradiation of skin enhances the Th2 responses characteristic of asthma, but instead can reduce them.

UV-induced suppression of murine asthma models

To our knowledge, only three groups have studied the effects of UV on allergic airway responses. In the experiments of Van Loveren and colleagues,^{10,20} BALB/c mice were given 2.2 kJ m⁻² UVB on 4 consecutive days before sensitisation to OVA by 7 intraperitoneal injections of 10 µg OVA every second day. Four weeks later, the mice were challenged 8 times on consecutive days with OVA aerosol.^{10,20} The results of assays of immunity were contradictory with increased IL-10 produced by mitogen-stimulated spleen cells from UV-irradiated mice. In contrast, levels of circulating OVA-specific IgE and IgG1 were reduced in the UV-treated mice. When the trachea of the mice were placed in an organ bath connected to an isometric transducer for a crude measurement of hyper-responsiveness, airway reactivity *in vitro* was reduced. A further research group studied the effects of UVB-irradiation (8 kJ m⁻²) of BALB/c mice before 4 involuntary aspirations of the fungal extract, *Metarhizium anisopliae*. Eosinophil numbers in lung lavage fluid and serum total IgE levels were reduced in the UVB-irradiated mice.²¹ Both these studies provided a framework upon which our more detailed studies of the effects of UV on models of experimental allergic airways disease were designed (Table 1).

Ovalbumin as allergen

In our first study,²² BALB/c mice were sensitised intraperitoneally to OVA (20 µg) with the adjuvant alum (4 mg). As stated above, both the strain of mouse and the adjuvant ensured a strong Th2-bias in the immune response to OVA. After 14 days, the mice were boosted with the same amounts of OVA with alum. After 21 days the respiratory system of the mice was challenged with aerosolised OVA and after 24 h significant experimental allergic airways disease was detected. Responses in three respiratory tissue sites were studied. Airways hyper-responsiveness is an assay of airway resistance to the bronchoconstrictor, methacholine, and provides a function associated with immune cells at the airway mucosa. In the bronchoalveolar lavage fluid, cells and cytokine levels were measured. For cells recovered from the airway-draining lymph nodes, their responsiveness *in vitro* to the sensitising allergen was examined. If the mice were irradiated with 8 kJ m⁻² UV (approximately 4× minimal oedema dose) three days prior to OVA sensitisation, all three readouts characteristic of asthmatic disease were significantly reduced. These included a reduction in airways



Prue Hart

the systemic immunomodulatory effects of UVB irradiation of skin. Her research also focuses on vitamin D control of immunological disease.

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Table 1 Reduced allergic airways disease in UV-irradiated mice

	Allergen	UVB protocol	Cell proliferation/ cytokine production	IgG1/IgE	Broncho-alveolar lavage fluid	Tracheal airway hyper-responsiveness
Van Loveren <i>et al.</i> ^{10,20}	OVA	×4 days, 2.2 kJ m ⁻²	↑ IL-10 by mitogen-stimulated spleen cells	↓ (OVA-specific)	Not done	↓ (Airway reactivity <i>in vitro</i>)
Ward <i>et al.</i> ²¹	<i>Metarhizium anisopliae</i>	×1, 8 kJ m ⁻²	Not done	↓ Total IgE	↓ Eosinophils	Not done
McGlade <i>et al.</i> ²²	OVA	×1, 8 kJ m ⁻² (also 2 kJ m ⁻²)	↓ OVA-specific proliferation by airway draining lymph node cells, ↓ IL-4, ↓ IL-5, ↓ IL-10	ns (OVA-specific)	↓ IL-5, ↓ eosinophils, ↓ neutrophils	↓ (By low-frequency forced oscillation technique <i>in vivo</i>)
McGlade <i>et al.</i> ²⁷	Papain	×1, 4 kJ m ⁻² (also 2 kJ m ⁻²)	↓ Papain-specific proliferation by airway-draining lymph node cells, ↓ IL-4, ↓ IL-5, ↓ IL-10	ns (Papain-specific)	↓ IL-5	Not detectable in C57BL/6 mice

ns: no significant change.

hyper-responsiveness to levels measured in naïve unsensitised mice.²² With a single aerosol challenge, UV irradiation significantly reduced IL-5 levels in the bronchoalveolar lavage fluid.²² With 3 consecutive daily aerosol challenges with OVA, UV also reduced by ~50% the significant increases in bronchoalveolar lavage fluid of eosinophils and neutrophils. The proliferation *in vitro* by single cell suspensions from the airway-draining lymph nodes of UV-irradiated, OVA-sensitised and OVA-challenged mice was significantly reduced.²² The levels of the Th2 cytokines, IL-4, IL-5 and IL-10, in the supernatants were reduced but not those of the Th1 cytokine, interferon- γ .²² Not only were 3 significant responses of asthma in different compartments of the respiratory system examined but, unlike the previous studies, allergen-specific responses by immune cells from the draining nodes, not spleen,^{10,20} were analysed. The lung function measurements were also completed with a low-frequency forced oscillation technique that can dissect responses to methacholine partitioned into components for the airways and lung parenchyma. As in asthma, in this OVA-induced model, only responses of the central airway were detected and were reduced in the UV-irradiated mice. OVA-specific IgE titres 3 weeks after initial UV-irradiation and OVA-sensitisation were reduced by ~25% in the UV-irradiated mice. It is hypothesised that with time and further OVA aerosol exposures, the UV effect may become significant.

The effect of UV irradiation of skin on immune responses to OVA can also be demonstrated at the airway mucosa, and not just in the airway-draining lymph nodes. From a mouse trachea, approximately 5×10^5 cells can be isolated. Of these, approximately 4% are T lymphocytes, 4% are B lymphocytes and 2% have been characterised as Class II+CD11c+ dendritic cells. Upon OVA exposure either by aerosol before trachea harvest, or by exposure *in vitro*, tracheal cells from UV-irradiated, OVA-sensitised and -challenged mice incorporated approximately 60% of the ³H-thymidine measured in tracheal cells from non-irradiated, OVA-sensitised and -challenged mice (Fig. 1); this assay provided a direct measure of OVA-induced proliferation. As we are unable to characterise by surface marker expression the cells that are sensitised to OVA, functional studies are ongoing to determine the basis of reduced OVA-induced proliferation by tracheal cells

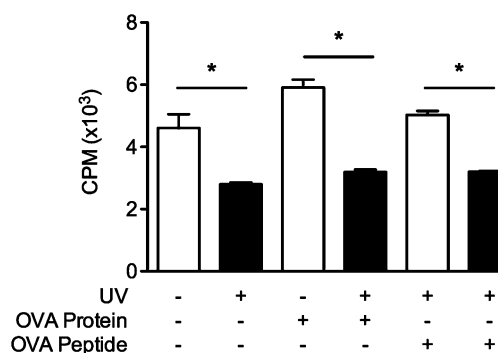


Fig. 1 Proliferation by tracheal cells from OVA-sensitised and -challenged mice, with or without UV exposure (8 kJ m⁻²) before sensitisation. Tracheal cells from mice in a representative experiment (10^5 cells per well) were cultured with or without OVA protein (100 μ g ml⁻¹) or OVA peptide (1 μ g ml⁻¹) for 96 h. Bars show mean + SEM, $n = 6$ replicate cultures. * $p < 0.05$.

from UV-irradiated mice. Importantly, the UV-regulatory effects were detectable at the mucosal surface.

In many experimental models, UVB can suppress immune responses when the irradiation is performed before challenge, as well as before sensitisation. Further, similar mechanisms may be involved.²³ As an example of UV-modulating immune responses post-sensitisation, in humans UVB can suppress hyper-reactivity to metals such as nickel, or to tuberculin purified protein derivative, in the skin of pre-sensitised or Mantoux-positive volunteers, respectively.^{24,25} Further study is required to define the regulatory effects of UV irradiation of skin on established allergic airways disease. In the results in Fig. 2, mice were irradiated with UV either 3 days before or 2 days after OVA sensitisation. The proliferation (again measured by ³H-thymidine incorporation) by single cell suspensions from the airway-draining lymph nodes harvested 24 h after OVA aerosol challenge was investigated. When the irradiation was delayed until after sensitisation, expression of allergic airways disease was still very significantly reduced. Delivery of UV during different phases of this experimental model of allergic airways disease is currently under further investigation.

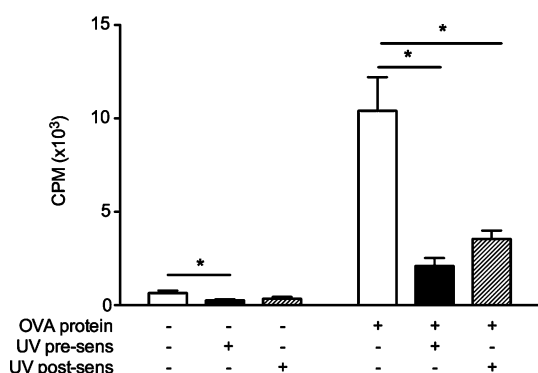


Fig. 2 OVA-induced proliferation by cells from the airway-draining lymph nodes of OVA-sensitised and -challenged mice. Mice were administered 8 kJ m^{-2} UV either 3 days before OVA sensitisation (pre-sens), or 2 days post-sensitisation (post-sens). Cells (10^5 cells per well) were cultured with or without OVA protein ($100 \mu\text{g ml}^{-1}$). Bars show mean + SEM, $n = 6$ replicate cultures. * $p < 0.05$.

Papain as allergen

In studies using an alternative allergen, C57BL/6 mice were sensitised to papain intranasally in the absence of an adjuvant. Papain is a cysteine protease homologue of Der p1, the dominant allergen from house dust mites.²⁶ Mice were sensitised intranasally to papain ($1 \mu\text{g}$) daily for 5 days, and similarly boosted after a further two weeks. Another 2 weeks later, mice were challenged intranasally with $100 \mu\text{g}$ papain on 3 consecutive days. This protocol caused significant inflammation of the airways. There was significantly increased numbers of neutrophils and eosinophils in the lung lavage fluid and increased reactivity to papain by the large number of immune and inflammatory cells that locate to the airway-draining lymph nodes. Further, in response to papain, substantial levels of Th2 cytokines (IL-4, IL-5 and IL-10) were produced, but not the Th1 cytokine, interferon γ . UV irradiation of the mice significantly reduced these inflammatory responses to papain.²⁷

Amounts of UV required to regulate experimental allergic airways disease

A summary of reports of reduced allergic airways disease in UV-irradiated mice is shown in Table 1. Only a single delivery of oedematous UV irradiation was required to regulate the inflammatory responses in the airways to OVA and papain.^{22,27} In fact, UV radiation as low as one minimal oedematous dose (2 kJ m^{-2}) suppressed responses by airway-draining lymph node cells to OVA in BALB/c mice, and to papain in C57BL/6 mice.^{22,27} Our studies suggest that it is not the inflammatory process *per se* in UV-irradiated skin that regulates responses to allergen. These are realistic amounts of UV radiation and are equivalent to less than 10 min in the noonday sun in Perth in summer (31°S). It is notable that these are studies of the consequence of a single exposure to UV irradiation. It is not clear whether multiple exposures to UV irradiation will have a greater or a different effect.

In our studies, the mice were irradiated with FS40 sunlamps (Westinghouse Corp., Pittsburgh, PA) emitting a broad band of UV, 250–360 nm, with 65% of the output in the UVB range

(290–315 nm). As UVC ($<290 \text{ nm}$) was screened out by polyvinyl chloride filters, the mice also received some UVA irradiation (315–400 nm). Additional studies are required to determine the relative contribution of the UVA irradiation to regulation of experimental allergic airways disease. UVA may itself be immunoregulatory as suggested in a study of OVA sensitisation with the adjuvant, saponin, and measurement of delayed-type hypersensitivity responses.²⁸ In that study using the same antigen as in one of our studies of allergic airways disease,²² UVA caused a bi-phasic dose response with intermediate but not low or high doses of UVA causing primary immunosuppression. However, in other studies, UVA can modulate the regulatory effects of UVB.^{29,30}

Mechanisms of UV-reduction of experimental allergic airways disease

The mechanisms by which immune responses to antigens administered to UV-irradiated sites are reduced (*local* immunosuppression) are relatively well defined.^{1,2} However, in the studies of allergic airways disease, the experimental allergens were administered to sites distant to the UV-irradiated skin and are therefore not processed and presented to T lymphocytes by UV-damaged antigen presenting cells. Our published studies suggest that mast cells at the UV-irradiated site are essential to the mechanisms by which UVB radiation causes systemic immunomodulation in both mice and humans.^{31,32} We hypothesise that mast cell products affect antigen-presenting cells at distant sites. In turn, these antigen-presenting cells (for example at the airway mucosa) stimulate an altered T cell response.

Two pathways in UVB-irradiated skin that initiate mast cell degranulation have been identified. These studies have confirmed that sensory neurons in skin are essential in UVB-induced systemic immunomodulation *via* both *cis*-urocanic acid (*cis*-UCA),³³ and nerve growth factor.³⁴ *cis*-UCA is formed from its less soluble isomer, *trans*-UCA upon UVB-irradiation of skin. *trans*-UCA (deaminated histidine) is located superficially in the *stratum corneum*. An antibody to *cis*-UCA neutralises the immunosuppressive effects of UVB in many experimental models.³⁵ In support of functional studies, the prevalence of dermal mast cells can determine the susceptibility of different mouse strains to UVB- and *cis*-UCA-induced systemic immunosuppression.^{31,35} Mast cell migration to draining nodes may also contribute to the immunomodulatory effects of UV.³⁶

Histamine is an important product of UV-activated, degranulating mast cells and can co-operate with *cis*-UCA for activation of keratinocytes for production of immunosuppressive prostaglandin E_2 .³⁷ Most immune cells express histamine receptors and histamine is involved in acute inflammatory processes. Histamine plays a role in the oedema, vasodilation, contraction of smooth muscle, up-regulation of adhesion molecules and hypersecretion of mucus associated with the allergic asthmatic reaction. The role of histamine in UV-suppression of experimental allergic airways disease was investigated by a study of the effect of UV irradiation on papain-induced airways inflammation in mice deficient in histamine type 1 and histamine type 2 receptors. Histamine working through the histamine type 1 receptor was important for modulation of papain-induced respiratory inflammation.²⁷ Histamine working through the histamine type 2 receptor, presumably on T lymphocytes,

was also involved in the mechanism by which UV suppressed papain-induced respiratory airways disease.²⁷ Collectively, these experiments implicate histamine-releasing mast cells in the afferent arm of UV-control of allergic airways disease.

In our published studies to determine how UV was immunomodulatory,²² single cell suspensions from the airway-draining lymph nodes of UV-irradiated mice harvested 24 h post-OVA-aerosol challenge were transferred into naïve mice. The transferred cells reduced subsequent sensitisation to OVA in the recipient mice. The cell transfer was allergen specific in that airway-draining lymph node cells from UV-irradiated, OVA-sensitised mice regulated sensitisation to OVA in recipient mice, but not sensitisation to β -lactoglobulin.²² The immunoregulatory effects of UV were also transferred by intravenous injection of airway-draining lymph node cells from UV-irradiated, papain-sensitised and -challenged mice into naïve mice subsequently sensitised to papain.²⁷ Although we await formal experiments, UV-induced T regulatory cells, generally identified as CTLA-4-expressing CD4+CD25+ cells,³⁸ may be responsible. It is notable, however, that not all effects of UV are due to induced regulatory cells. In a recent study *in vivo* of Th1-driven contact hypersensitivity, sub-erythral doses of UVB suppressed immunity by inhibiting effector and memory T cells without inducing regulatory CD4+ T cells.³⁹

Does UV-induced vitamin D contribute to UV-modulation of experimental allergic airways disease?

The involvement of vitamin D in UV-regulation of experimental asthma models remains an unanswered question, and may vary depending on the number of exposures to UV radiation. Vitamin D would be an exciting therapeutic if it controlled allergic airways disease and avoided the carcinogenic effects of UVB exposure. Humans obtain approximately 90% of their vitamin D by skin exposure to UVB wavelengths in sunlight. 7-Dehydrocholesterol in skin is converted by UVB exposure to vitamin D, which is then hydroxylated at the 1 α - and 25- positions to form the hormonally- and immunologically-active form of vitamin D, 1,25-dihydroxyvitamin D3.⁴⁰ Low vitamin D status (generally measured as serum 25-dihydroxyvitamin D3 levels) has been associated with poor lung function,⁴¹ and polymorphisms in the vitamin D receptor have been linked with asthma incidence.⁴² Animal studies have also generally supported the concept that chronic vitamin D administration can reduce asthma symptoms in murine experimental asthma models.^{43,44} In fact, some scientists hypothesise that childhood asthma may be a consequence of vitamin D deficiency.⁴⁵

Our studies suggest that a single irradiation with an oedematous amount of UV can suppress murine asthma models.^{22,27} However, in response to 8 kJ m⁻² UV irradiation to shaved dorsal skin, we were not able to measure significant changes to circulating 25-OH vitamin D3 levels after 1, 2 or 3 days. Previous studies from this laboratory have shown that a single topical application of 1,25-dihydroxyvitamin D3, at a concentration measured in the fluid of experimental blisters raised on skin subsequently exposed to UV-irradiation,⁴⁶ can enhance the regulatory activity of CD4+CD25+ cells isolated from the lymph nodes draining

the skin site of 1,25-dihydroxyvitamin D3 application. These cells were more suppressive in proliferation assays *in vitro*. Also, when these cells were transferred by tail vein into new mice which were subsequently exposed on ventral skin to the experimental hapten, dinitrofluorobenzene, and challenged on ear skin with the hapten, a contact hypersensitivity response (or recall response) was significantly suppressed.⁴⁷ We hypothesise that a single application of 1,25-dihydroxyvitamin D3 to skin can enhance the suppressive ability of CD4+CD25+ cells for regulation of the immunological processes of allergic airways disease. However, this has yet to be formally shown particularly as 1,25-dihydroxyvitamin D3 may stimulate CD4+ cells to express only skin-homing receptors.⁴⁸

Some evidence that vitamin D may be involved in the mechanism by which UV regulates immune responses comes from studies of expression of the ligand of receptor activator of nuclear factor κ B (RANKL) on keratinocytes following UV-irradiation of skin.⁴⁹ T lymphocytes express RANK and an interaction with RANKL-expressing keratinocytes has been linked with induction in skin of greater numbers of CD4+CD25+ T regulatory cells. More recently it was shown that the vitamin D3 analogue, calcipotriol, could induce the expression of RANKL on keratinocytes.⁵⁰

If vitamin D is an intermediary by which UV-irradiation can regulate allergic airways disease, 1,25-dihydroxyvitamin D3 may contribute to mast cell activity. Indeed, 1,25-dihydroxyvitamin D3 has been reported to both stimulate mast cell maturation⁵¹ as well as enhance their degranulation.⁵²

Comment on the atopic march from skin to the respiratory system

Atopic dermatitis is often the initial step in the so-called 'atopic march'. It has been shown that 50% of patients with moderate to severe atopic dermatitis develop asthma later in life.⁵³ Contrary to our experimental studies of regulatory rather than disease-promoting skin-derived mediators, this observation suggests a direct link between inflammation of the skin and development of allergic airways disease. Recent studies have shown that thymic stromal lymphopoietin overproduced by keratinocytes triggers atopic dermatitis,⁵⁴ followed by a progression from skin disease to asthma.^{55,56} It is notable that in one study topical application of an analogue of vitamin D3 triggered skin inflammation and thymic stromal lymphopoietin production; however the amounts of vitamin D applied were 10-fold higher than those tested in the studies of Gorman *et al.* described above,⁴⁷ and the vitamin D was applied every other day for 21 days.^{55,56} These studies however support the concept that biological changes in the skin can signal to the respiratory system for control, both positively and negatively, depending on the nature and strength of change, of allergic airways disease.

Relevance of UV exposure protecting against allergic airways disease in mice

This review has described regulatory effects of UV irradiation of skin on experimental allergic disease in mice. The amounts of UV given are realistic and can be obtained with 10 min of summer sun exposure in Perth (31°S). Our studies do not suggest that inflammation induced in the skin due to the single administration

of oedematous UV contributes to the mechanisms of regulation. Instead, 1,25-dihydroxyvitamin D₃, mast cells and histamine type 2 receptors may contribute to the afferent arm of the mechanism by which UV is regulatory. The histamine type 1 receptor may contribute to expression of the effector arm of allergic airways disease but not the mechanism of UV regulation. With the mechanisms of control by UV and its intermediaries better understood, we may be able to harness the positive regulatory effects and avoid the carcinogenic effects of UV skin exposure. The studies in mice complement anecdotal reports that holidays at the beach or in the mountains, where there is increased UV exposure, are beneficial for the development and expression of asthma symptoms. Further, an advancement in knowledge of how UV regulates allergic airways disease may be beneficial for control of other immune systemic diseases, for example multiple sclerosis, which have been linked strongly with vitamin D status.⁵⁷

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