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Title: ASTHMA – comparing the impact of vitamin D versus UVR on clinical and immune parameters

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Abstract

The incidence of asthma has increased markedly since the 1960s and is currently estimated to affect more than 300 million individuals worldwide. A number of environmental factors are implicated in asthma pathogenesis, one of which is vitamin D. Vitamin D deficiency is a global health concern and has increased in parallel with asthma incidence. Epidemiological studies report links between low vitamin D status, assessed as circulating levels of 25-hydroxyvitamin D₃, with asthma incidence, severity, exacerbations and responses to treatment. This has led to clinical studies to test whether increasing the levels of vitamin D improves asthma management. Despite being highly variable in dosing regimens, design and outcomes, meta-analyses suggest overall positive outcomes with respect to reduced asthma exacerbations and steroid requirements. The primary mechanism for increasing vitamin D levels in the body is through exposure of the skin to Ultraviolet B (UVB) component of Ultraviolet radiation (UVR), most commonly from sun exposure. However, only a limited number of studies investigating the impact of UVR on the asthmatic response have been performed; these generally report on the impact of latitude as a surrogate of sun exposure, or address this in animal models. To the best of our knowledge no comprehensive trials to assess the impact of UVB irradiation on asthma outcomes have been performed. Within this review we discuss observational and clinical studies in this field, and innate and adaptive immune mechanisms through which UVR and vitamin D may impact respiratory health, and asthma. We highlight the heterogeneity of asthmatic disease, which is likely to impact upon the efficacy of interventional studies, and briefly overview more recent findings relating to the impact of vitamin D/UVR on the development of asthma.

Key words: vitamin D, ultra violet radiation, supplementation, asthma.

Non-standard abbreviations: 25(OH)D₃ – 25-hydroxyvitamin D₃; MED - minimal erythematol dose UVB – Ultraviolet B radiation, Ultraviolet radiation (UVR).

Heterogeneity of Asthma

Asthma is the most common chronic inflammatory disease of the lungs. It is estimated to affect more than 300 million people globally, causing a significant impairment in their quality of life and a major burden on healthcare resources. The incidence varies widely between countries, and in the UK alone, there are more than 5 million asthma patients, of which over 1 million are children [1, 2]. Originally considered a disease of high-income countries, asthma prevalence is now estimated to be increasing fastest in low- and middle-income countries, highlighting the importance of environmental influences. Diet, exposure to pollution and allergens, respiratory infections, as well as vitamin D deficiency, the focus of this review, are just some of the risk factors associated with the increased prevalence and exacerbations of asthma. Vitamin D is traditionally known for its effects on bone and mineral homeostasis, but it is now known to also have profound immunomodulatory properties [3, 4]. UVB irradiation is crucial in enabling the synthesis of vitamin D within the body, but vitamin D is also, albeit less commonly, obtained through diet via the ingestion of foods such as fatty fish and offal, or through the use of vitamin D supplements.

Episodic chest tightness, wheezing and breathlessness typify asthma. Clinically asthma is characterised by airway hyper responsiveness (AHR), bronchial constriction and mucosal hyperplasia that are, in most cases, reversible either spontaneously or with treatment. Despite similar symptomatic presentation, asthma is a heterogeneous disease with distinct endotypes. Endotype division is based on clinical and physiological factors, as well as underlying immunopathology, all areas of on going debate [5, 6]. Clinical variables in asthma presentation include the age of onset, drug responsiveness, obesity and severity of symptoms including number of hospitalisations and respiratory infections. Further complexity is added by the fact that the underlying pathological mechanisms may not be stable throughout an individual's life, altered for example by environmental factors such as air pollution [7].

One of the most common immunological divisions of asthma is non-atopic versus atopic; the latter is characterised by elevated levels of IgE antibodies to environmental allergens and is true for the majority of children and approximately 50% of adults [5, 6]. Asthma as an allergic disease implies the involvement of Th2 cells and Th2-associated cytokines IL-4, IL-5,

IL-13, mast cells and eosinophils. This Th2-dominant form of asthma is typically well controlled with β -adrenergic agonists and/or corticosteroids. There are nonetheless approximately 5-10% of asthmatics who show poor, or no, clinical benefit from corticosteroids despite taking very high doses of inhaled and oral corticosteroids, yet do remain susceptible to their side effects (steroid-refractory, SR) [8]. Disease in these individuals is often severe and is frequently characterised by high levels of IL-17A and neutrophilia [9]. Improved understanding of asthma endotypes and their distinct underlying immunopathological mechanisms will enable development of better-targeted and efficient therapeutics with reduced side effects, including UVR/vitamin D.

The effects of UVR exposure and vitamin D supplementation

Vitamin D supplementation and exposure to UVB irradiation do not have identical biological effects [10]. This review will discuss the impact of vitamin D status, as determined by serum 25-hydroxyvitamin D3 (25(OH)D3), on the prevalence and severity of asthma, and experimental evidence of how vitamin D modulates immune mechanisms associated with disease pathology; where known we will highlight data on the effects of UVR on these parameters.

The impact of latitude on asthma

Vitamin D levels are most commonly regulated through UVB exposure of skin, which is affected for example by latitude, cloud density, solar zenith angle, ozone and pollution levels [11]. Epidemiological studies suggest that on average, our exposure to sun has decreased due to a more sedentary lifestyle with increased time spent indoors and a greater awareness of the detrimental impact of UVR exposure, including photoaging and carcinogenesis (reviewed in Young, 2006 [12]). Alongside this, evidence exists for a positive correlation between asthma incidence and latitude gradient; for example, certain western, industrialised countries that are furthest away from the equator such as New Zealand, parts of Australia and the UK account for some of the highest numbers of asthma cases worldwide [1]. Indeed Krstić reported that a 10° change in latitude from southern to northern regions of the USA and Australia were associated with a 2% increase in the frequency of asthma in adults [13]. However, Hughes *et al.* identified that this latitude gradient across Australia was ablated after adjusting for average daily temperature [14]. In a

similar vein, a Spanish study identified a latitude gradient between the coastal zones and interior of Spain, but inferred that this may be heavily influenced by regional relative humidity which was negatively associated with asthma rather than with latitude [15]. Therefore, the association between latitude and asthma may be at least in part due to other climate factors or might reflect a variation in aeroallergens due to these influences. The effect of latitude is further compounded by season, since at the extremes of latitude there is insufficient UVB intensity in winter and early spring months for adequate synthesis of vitamin D to occur [16] accompanied by a period when asthma exacerbations are more frequent.

The subsequent review in this series addresses the impact of UVR/vitamin D in allergic disease. Understanding associations with allergen-triggered disease include additional complexities such as the impact of warmer climates on increased allergens e.g. pollens. Although several studies report on the effect of latitude, UVR and/or vitamin D status on hayfever and asthma (e.g.[14]), future studies that specifically compare or review available data on distinct asthma endotypes, including allergic vs. non-allergic asthma, will be of interest.

Whilst many studies use UVR as a proxy for vitamin D status this conclusion does not always take into account lifestyle behaviours influencing actual UVR exposure or the potential additional effects of both UVA and UVB exposure to the body. For example, it is plausible that severe asthmatic patients spend less time outside due to their condition and are therefore exposed to less sunlight necessary for natural vitamin D synthesis, although the limited available evidence does not support this theory [17], and certainly not as a full explanation. Furthermore, in certain regions of the world latitude is likely to be less reflective of vitamin D status due to genetic and cultural differences which include, but are not limited to, dark skin pigmentation, excessive clothing, restricted outdoor activity, high levels of atmospheric pollution, and low dietary intake of vitamin D and calcium.

Associations between serum 25(OH)D and asthma

Irrespective of the correlations with latitude there is a clear association between low serum 25(OH)D₃ levels and asthma. A role for vitamin D in maintaining pulmonary health was

highlighted in 2005 when a positive correlation was observed between serum 25(OH)D3 and FEV₁ [18]. As we and others have recently summarised, higher levels of vitamin D are generally associated with a lower risk of asthma incidence and exacerbations, reduced asthma severity and hospitalisations, improved response to therapy and a lower incidence of respiratory tract infections, a primary trigger for asthma exacerbations (extensively referenced in [4, 19]); see also [20, 21]). Additional studies regarding the potential of vitamin D to reduce the incidence of asthma through effects *in utero* and foetal development are discussed further below.

UVR intervention studies in asthma

We are unaware of any interventional studies that have directly assessed the impact of UVR treatment on asthma to date. However, a report of 69-year old twins, both of whom presented with severe psoriasis, neutrophilic asthma and vitiligo vulgaris, may suggest some benefit. Only one twin consented to additional narrowband ultraviolet B treatment, and only this twin was reported to show clinical benefit of UVR on their psoriasis, vitiligo and neutrophilic asthma, alongside a decrease in serum TNF α , IL-1, IL-6, IL-8, IL-17A and IL-12 [22]. Phototherapy was administered three times weekly for three months, and it is thus likely that vitamin D levels were enhanced, although this was not reported. Th1/Th17 responses have been implicated in the pathology of severe neutrophilic asthma [4, 9, 23], and as discussed further below, vitamin D reduces Th1/Th17 responses making it plausible that the UVR treatment may act via vitamin D to beneficially influence this immune profile. Whilst the evidence from the Malerba *et al.* study must remain anecdotal, further clinical studies to address the relationship between human airway function and asthma with UVB exposure are awaited with interest.

Oral vitamin D supplementation in asthma

Studies of vitamin D supplementation in the asthma field are emerging. The design of these studies is highly variable, differing for example in the supplementation regimen (dose, vitamin D2/D3, bolus vs. intermittent, duration), inclusion criteria, outcome measures and importantly baseline vitamin D status [19]. These trials increasingly support the concept that vitamin D is safe, and do not report adverse effects. However, many studies fail to demonstrate significant effects on the pre-defined primary outcome. Nonetheless many

studies report trends towards improved lung function, reduced asthma exacerbations, disease severity, and/or corticosteroid dependence following supplementation [4, 24-29]. For example, the largest vitamin D supplementation study in asthma to date is the VIDA (Vitamin D Add-on Therapy Enhances Corticosteroid Responsiveness in Asthma) trial, in which adults receiving inhaled corticosteroids were supplemented with daily 4000 IU vitamin D3 after an initial 100,000 IU bolus or placebo. Although the authors reported no significant difference on their primary outcome, the time to first asthma treatment failure, the vitamin D supplemented group required a significantly lower average inhaled corticosteroid dose and there was a trend ($p=0.05$) towards reduced overall exacerbation rate [24]. However, in both arms of the study only 50% or less of the participants were vitamin D deficient ($<20\text{ng/ml}$) at baseline. It may be that vitamin D has a significant protective effect only when treatment is given to deficient individuals to bring levels back to sufficiency. This was recently highlighted in a study of chronic pulmonary disease (COPD) where a significant protective effect of vitamin D supplementation was only established in patients with baseline $25(\text{OH})\text{D}_3$ of $<50\text{nmol/L}$ [30].

A lack of effect in some studies may be a result of limited patient stratification, such as individuals being vitamin D sufficient at the start of the study. The inconsistency of results may also be in part attributable to a nonlinear relationship with vitamin D intake and serum levels [31] [19], with Grant *et al* highlighting a potential U shape curvilinear relationship between vitamin D and health outcomes. Additionally, polymorphisms may affect vitamin D responsiveness since various polymorphisms within the VDR and other vitamin D pathway genes are associated with an elevated asthma risk e.g. [32, 33].

Alongside knowing what an ideal serum $25(\text{OH})\text{D}$ level is, deciphering how much supplementation is required, and the best mode of delivery, to sufficiently increase serum $25(\text{OH})\text{D}$ requires clarity. One study determined that when baseline serum $25(\text{OH})\text{D}$ was below 75 nmol/L , serum $25(\text{OH})\text{D}$ could be increased by 2 nmol/L for every microgram (40 IU) of vitamin D3 given [34]. However the effect of vitamin D supplementation on serum $25(\text{OH})\text{D}$ may differ considerably between individuals [35]. Of note, bolus doses have failed to stabilise levels of $25(\text{OH})\text{D}$ when too spread out resulting in peaks and troughs [36], with potential implications for therapeutic efficacy [27]. Importantly, to date, no major side

effects of vitamin D supplementation have been knowingly reported in clinical asthma studies.

Taken together, there is currently insufficient evidence to confidently state that vitamin D should or should not be recommended as an adjuvant therapy for asthma. However, at least one recent systematic review suggests that vitamin D may reduce the risk of severe asthma exacerbation and healthcare use [37]. Importantly, these data increasingly suggest that vitamin D is safe. More mechanistic studies and well-designed large clinical studies based on research outputs, and employing patient stratification, should provide further clarity on clinical efficacy, patient stratification, and underlying biological mechanisms. The issue of optimal mode of delivery remains an important question, with current studies focussing on vitamin D supplementation; the issue of how UVB irradiation compare to these regimes, and whether any reported UVB effects are dependent or independent of vitamin D synthesis, has not been extensively addressed.

Mechanisms by which vitamin D and UVR/UVB may influence airway function and asthma pathology

Evidence that structural cells in the airways, and many immune cells such as dendritic cells (DC) and macrophages synthesize the active form of vitamin D ($1,25(\text{OH})_2\text{D}_3$), particularly during inflammatory responses [38, 39], imply a role for local synthesis in the modulation of innate and adaptive immune pathways in the airways. More specifically, CYP27B1 is reportedly expressed by epithelial cells, macrophages and DCs, which are capable of producing 10^{-9} to 10^{-8}M $1,25(\text{OH})_2\text{D}_3$ from an excess of $25(\text{OH})\text{D}_3$ [38, 40-45]. UVB exposure of the skin to increase circulating levels of $25(\text{OH})\text{D}_3$ will presumably impact the potential for such local synthesis. Additionally, the VDR is expressed in essentially all cells of the immune system, either basally or upon activation. Vitamin D is thought to influence approximately 3% of the human genome [45], and vitamin D response elements exist across many lung-, asthma- and immune-associated genes [33]. Together these data have fuelled the search to understand the biological effects of vitamin D that may explain the observed associations of vitamin D with airway health.

Epidemiological studies in humans suggest a potential relationship between UVR exposure and the prevalence of asthma. Yet, to date, few studies have investigated the systemic effects of UVR exposure on asthmatic disease, or the role of vitamin D in any UVR mediated effects, including alterations in vitamin D status following UVR exposure. The majority of mechanistic data related to UVR derive from animal models. A comparison of the immunological effects of vitamin D and UVB was the subject of a comprehensive and more general review in 2011, which contrasted their effects in psoriasis, autoimmune, allergic and infectious disease [10]. Here mechanistic studies of the effects of vitamin D relevant to asthma, including the regulation of innate and adaptive immune functions, airway epithelium, smooth muscle cell remodelling and modulating responsiveness to asthma therapies, are discussed in the following section; where available data on UVR are compared.

Airway remodelling

Airway remodelling is a major feature of severe asthma, including increases in airway smooth muscle (ASM) mass, which is attributed to hyperplasia, hypertrophy and/or increases in extracellular matrix (ECM) [46]. Vitamin D treatment has been shown to inhibit hyperplasia, and the increased ASM reported in asthmatics has been shown to be opposed by vitamin D in both mice [47] and humans [48-50]. Foong *et al* showed that vitamin D deficiency in adult female mice caused AHR, which was accompanied by an increase in ASM mass, smaller lung volume and altered lung structure. although male mice appeared more resistant to associated changes in lung volume and structure [47]. The effects of vitamin D on airway remodelling have been reviewed elsewhere, however overall these studies suggest the impact may be greatest *in utero* and childhood [48, 49].

Epithelial cells

Epithelial cells are the first line of defence against the exposure of the airways and lung to inflammatory stimuli and antigens. As such, it is unsurprising that activation of the epithelium is one of the major characteristics of asthma, and that epithelial-associated genes are the most commonly upregulated in asthma GWAS studies [51]. Primary human bronchial epithelial cells (HBECs) express CYP27Ba and can convert 25(OH)D into

1,25(OH)₂D₃ [38]. Vitamin D can improve epithelial barrier integrity [52] and reduce production of pro-inflammatory cytokines by virally infected primary HBECs [53] [19].

Antimicrobial peptides and pathways

Antimicrobial peptides (AMPs) are produced by multiple cell types, including epithelial cells, and form an important line of defence against pathogens. Two principle families of AMPs exist, the cathelicidin peptide hCAP18/LL-37 and β -defensins which enhance microbe killing through disruption of bacterial and viral membranes, although broader actions of AMPs on skin barrier integrity and adaptive immunity have also been proposed [54]. Vitamin D increases the expression of AMPs such as cathelicidin in epithelial cells and monocytes [53, 55, 56], as well as promoting autophagy and autophagosome activity [56] [57]. Exposure to UVR has also been shown to increase production of AMPs (reviewed in [54]). For example, UVB upregulated expression of hCAP18/LL37 in human skin [58]; this effect was driven by UVB radiation specifically, and UVA exposure did not increase AMP expression after a single low dose exposure of 1 MED UVR. This effect was correlated with an increase in vitamin D receptor expression. Increased production of these AMPs potentially inhibits microbial replication and reduces the susceptibility to respiratory viral infections [59], a major trigger of asthma exacerbations. It is highly plausible that induction by vitamin D of antimicrobial pathways explains, at least in part, the reported effects of vitamin D in reducing asthma exacerbations, and represents a common mechanism that links vitamin D and UVB. These data are supported by additional studies without an asthma-specific focus that also demonstrate statistically significant observational studies and RCT, without an asthma-specific focus, that also demonstrate statistically significant associations between vitamin D status and an increased risk of upper and lower respiratory tract infections [60, 61].

B cells

The *in vitro* and *in vivo* effects of vitamin D on B lymphocyte function were recently reviewed [62]. With respect to allergy and asthma, 1,25(OH)₂D₃ dampens production of IgE from peripheral human B cells and increases IL-10 production, thus switching cells towards a regulatory phenotype [63, 64]; IL-10-synthesising B cells also secreted IgG4 which is thought

to be protective in allergen desensitisation therapy [65]. Notably, UV irradiation of mice results in reduced IgE and IgG1 in mouse models of allergen-induced “asthma” [66].

Dendritic Cells (DC)

The scale and nature of the T lymphocyte response to antigen is dependent upon DC processing and presentation of antigen. Resting DCs are situated primarily in the airway epithelium and submucosa where they act as sentinels and take up inhaled antigens/allergens. Local, including epithelial-derived signals received by the DC determine whether effector or tolerogenic adaptive immune responses result [67]. Tolerogenic DCs are characterised by a decrease in the expression of MHC class II, maturation and co-stimulatory molecules, and decreased secretion of immunostimulatory cytokines. DC are both a source of active vitamin D₃, 1 α ,25-dihydroxyvitamin D₃ [41] and a target for its actions [68]. Exposure to 1,25(OH)₂D₃ generally promotes a more tolerogenic DC phenotype, in part by inhibiting myeloid (m)DC differentiation and maturation (reduced HLA-DR, CD40, CD80 and CD86), whilst upregulating expression of molecules involved in antigen capture in a dose-dependent fashion. In addition, 1,25(OH)₂D₃-treated DCs secrete less CCL17 and CCL22 and show reduced chemotaxis in a CCR7/CCL21-dependent manner; and pro-inflammatory and immunomodulatory cytokines such as IL-12 and IL-23, which deviate T cell responses towards Th1 and Th17 phenotypes respectively, are generally reduced. Exposure to 1,25(OH)₂D₃ increases expression of inhibitory co-stimulatory molecules such as ILT3 and PDL1, and anti-inflammatory IL-10 (reviewed in Mann [4]); Co-culture of naïve T cells with 1,25(OH)₂D₃-treated mDCs results in an enhanced frequency of Tregs, elevated IL-10 production, reduced proliferation, and dampened levels of the pro-inflammatory cytokines TNF α , IL-12 and IFN γ [68].

Langerhan's cells in the skin are a target of UVR exposure, resulting in their migration from the epidermis to local draining lymph nodes where they are reported to induce antigen-specific T regulatory cells (Treg) [66] [69]. This effect is not seen in animals in which Langerhan's cells were specifically deleted [69]. UVR exposure alters the antigen presenting ability and migration of skin-resident Langerhan's cells, as well as their effect on T cell homing [42]. *Ng et al*, demonstrated that UV irradiation of mice affected the function of bone marrow-derived CD11c(+) cells, even though these were distal to the site of

irradiation, providing evidence of systemic effects of local UV irradiation [70]. In order to dissect the effects of UVR mediated by vitamin D versus other mechanisms, two broad strategies have been employed; irradiation of vitamin D receptor (VDR) knockout animals, or using UVR wavelengths lacking vitamin D synthesis potential. UVR administration was shown to act in a similar manner to topical vitamin D to induce Tregs. However, mice genetically lacking the VDR still responded as robustly to UVR for Treg induction, implying both common and distinct effects of the two [71]. Thus as a generalization both UVR and vitamin D promote a more tolerogenic DC phenotype, however the mechanism(s) by which this occurs are unlikely to be identical. UVA exposure was shown to induce migratory dermal dendritic cells that possessed a tolerogenic phenotype with decreased CD80, CD86, CD83 and CCR7 expression alongside a reduced capacity to stimulate T cell proliferation [72]. However in a study of patients with atopic eczema, the overall migratory capacity of DC was reduced by high-dose UVA1 [73]. Dose appears to play an important role in these processes, the discussion of which is beyond the scope of the present review on asthma.

T cell activation

Upon TCR activation a naive CD4⁺ T cell can differentiate into a range of T helper (Th) subsets in the periphery that perform distinct functions and home to specific tissues. This process is dependent upon multiple factors including the local cytokine milieu, downstream cytokine receptor signalling mediated by signal transducer and activator of transcription (STAT) transcription factors and expression of subset-specific hallmark transcription factors (such as Tbet (Th1), GATA3 (Th2) and RORC2 (Th17)). The functions of these subsets are tailored to the resolution of distinct types of infections, however the roles of Th2 and Th17 have also been a major focus in the pathogenesis of human asthma.

Th2

The dominant form of asthma presents with a skewing towards a Th2 phenotype. This is characterized by the production of the Th2-associated cytokines, IL-4, IL-5 and IL-13, the production of IgE by B cells, and the differentiation and activation of mast cells and eosinophils. Together, these factors may drive the induction and maintenance of asthmatic pathology in some individuals as discussed above ('Heterogeneity of asthma'). As reviewed

in Lange et al. there are contradicting reports as to the immunomodulatory effect of vitamin D on Th2 responses, and depending on the experimental model, vitamin D either enhances or reduces the Th2 phenotype [74].

Independent groups have shown that that 1,25(OH)₂D₃ treatment *in vitro* promotes the differentiation of a Th2 population with increased IL-4, IL-5 and IL-10 production [75, 76]. However, there is also evidence that 1,25(OH)₂D₃ inhibits IL-4 transcriptionally in cord blood cultures [77]. *In vitro* studies have shown that 1,25(OH)₂D₃ reduces levels of Th2-driven IgE, and increases IL-10 production [4, 62, 78]. This is reflected in observational studies that have identified a correlation between low serum vitamin D levels and enhanced aeroallergen-specific IgE levels [50, 79], although elsewhere a non-linear relationship has been reported, with both extremes of low and high serum 25(OH)D₃ associated with increased IgE [80]. Likewise, UVB exposure has been reported to reduce allergen specific IgE levels in a OVA sensitised murine model [81], but may enhance Th2 responses in others eg. [82]. Thus, the effect of UVR and vitamin D in the context of Th2-mediated responses in asthma is not clear. *In vitro* vitamin D promotes other pathways such as expression of the decoy receptor soluble ST2 (sST2) in HBECs and CD4⁺ T cells, which are predicted to counteract the Th2 response by inhibiting IL-33 [83], as well as additional immunoregulatory mechanisms discussed below.

The effects of UVR irradiation have been studied in murine models of Th2-driven allergic airway inflammation or “asthma”. The model allergen ovalbumin (OVA) together with the adjuvant aluminium hydroxide stimulated asthmatic symptoms including antigen-specific IgE, airway hyper responsiveness, and inflammatory cytokines in the BALF [84]. McGlade *et al.* demonstrated that a single minimal erythral dose (MED) significantly reduced symptoms when given either before or after challenge with the allergen, however it is unclear whether these effects were mediated through vitamin D synthesis or more global UVR-mediated effects [85]. Reduced symptoms were accompanied by significant reduction in airway resistance in response to methacholine (a routine “asthma diagnostic test” in mice) as well as significantly lessened levels of IL-5 in bronchoalveolar lavage fluid. Similar data were obtained in a papain model of airway disease [66].

Th9 cells and mast cells.

IL-9 is the dominant cytokine produced by Th9 cells, a proposed T helper subset, that develops in the presence of IL-4 and TGF β . IL-9 plays an important role in the regulation of mast cells, central players in allergic and asthmatic responses. Vitamin D has been shown to potently inhibit IL-9 synthesis [86]. The effects of UVR/UVB, have not, to the best of our knowledge, been reported on Th9 cells. However, it has been shown that both vitamin D and UVR modulate the function of mast cells. Mast cell activation in allergy, classically through allergen cross-linking of surface bound IgE, leads to degranulation and the release of mediators such as histamine. Histamine and additional mediators increase vasodilation, secretion of mucous and contraction of smooth muscle within the airways, all hallmarks of allergic asthma. Human and mouse mast cells possess the ability to metabolise vitamin D locally, which results in the repression of IgE-mediated triggering of human and murine mast cells [87]. Several studies have shown that migration of mast cells to the skin draining lymph nodes is stimulated by exposure to solar simulated UVR in a dose dependent manner e.g. [88, 89]. Furthermore, it has been suggested that UVB-mediated systemic suppression of the contact hypersensitivity response is mast cell dependent [89]. Additionally, Biggs *et al*, reported that 1,25(OH) $_2$ D $_3$ induction of IL-10 production by cutaneous mast cells can contribute to suppression of inflammation and skin pathology at sites of chronic UVB irradiation, and this effect is lost in VDR knockout mast cells [90]. Mast cells appear to modulate immune responses from distant sites, with UVB radiation of skin inducing systemic immune tolerance to allergens (reviewed in [66]). Thus, evidence for the impact of UVR/Vitamin D on mast cells *in vitro* and in mouse models is of interest, however, such studies in human tissue or in intervention studies are limited.

Th17/Neutrophilic and steroid refractory asthma

Whilst asthma is known to be a predominantly Th2-mediated disease there is evidence of a severe asthmatic endotype presenting with high levels of the proinflammatory cytokine IL-17A in the sputum and bronchoalveolar lavage (BAL) fluid [4, 91]. Th17 cells are important for defence against fungal and bacterial lung infections at mucosal sites such as the airways, but in excess drive a neutrophilic airway inflammation. Patients with an IL-17A^{high} phenotype have a skewing to towards a Th17-mediated response, which is not controlled by

the use of glucocorticoids. Nanzer *et al.* identified a 7 fold increase in PBMC production of IL-17A in steroid refractory patients that was significantly inhibited by 1,25(OH)₂D3 *in vitro* in a glucocorticoid-independent manner [76]. Xystrakis *et al.* demonstrated additional steroid-enhancing properties of vitamin D in these patients. The capacity of dexamethasone to induce synthesis of the anti-inflammatory cytokine IL-10 in PBMC and T cell cultures was impaired in steroid refractory asthmatics, and addition of 1,25(OH)₂D3 to these cultures, or following 1-week of ingestion of 1,25(OH)₂D3 by 3 steroid refractory patients, restored this response to one akin to that of a steroid sensitive phenotype [92]; these data have since been validated and extended by a proof of concept study of 1 α ,25-dihydroxyvitamin D3 (calcitriol) treatment in steroid refractory asthma patients [23]. Similar steroid-enhancing properties of vitamin D are likely to exist in monocytes [93].

These effects of vitamin D on human Th17 responses in asthma are mirrored by reports that effective treatment of psoriasis with UVB was linked to reduced Th1- and Th17-associated cytokines [94-96]. In a single case report, similar effects were observed in neutrophilic asthma [22]. As described for Th17/neutrophilic asthma, late-onset eosinophilic asthma is often associated with a steroid resistant phenotype making it difficult to treat, and has also been reported to be reduced after oral vitamin D treatment [25]. In parallel, data from a murine model of allergic asthma show UVB treatment also reduced eosinophil and neutrophil number within bronchoalveolar lavage fluid (BAL) by approximately 50% [66, 84].

T regulatory cells

Regulatory T cells are crucial in the maintenance of immune homeostasis. They inhibit the function of effector T cells, antigen presenting and innate cells. Within the context of asthma they inhibit Th2 responses, airway inflammation and airway hyper responsiveness through direct cell-cell interactions and the production of soluble mediators such as IL-10 and TGF β . The adoptive transfer of CD4⁺CD25⁺ T cells has been shown to suppress hyper reactivity and allergic inflammation e.g. [97].

Vitamin D modulates various facets of Treg actions in a manner that is often dependent upon the environment [98]. 1,25(OH)₂D3 is reported to increase the frequency of human

CD4+Foxp3+ and CD4+IL-10+ cells *in vitro* with minimal co-expression [43, 78]. These *in vitro* observations are supported by studies showing that serum 25(OH)D positively correlates with the frequency of CD4+Foxp3+ T cells in the periphery and BAL, as well as levels of IL-10 in BAL [78, 99, 100]. Furthermore, 6 months of vitamin D supplementation significantly increased the ratio of Foxp3+:IL-17A+ CD4+ T cells in the peripheral blood of SLE patients [101]. Beyond IL-10 and Foxp3, 1,25(OH)₂D₃ also enhances expression of immunosuppressive CD200 [102] and the inhibitory co-stimulatory molecules CTLA-4 [98] and PD-1 [78].

The impact of UVR to influence Treg frequency and function has been studied in a number of immune-mediated conditions [10], and ones relating to Th2-mediated airway inflammation are discussed above in sections on “Dendritic cells” and “Th2 cells” and in two recent reviews [10, 66]. The above data on the effect of vitamin D to promote human Treg mirror evidence in mice that a single administration of topical 1,25(OH)₂D₃ enhanced the suppressive capacity of CD4+CD25+ cells from skin draining lymph nodes, albeit not in an “asthma” model. In this study the authors compared topical 1,25(OH)₂D₃ versus UVB-irradiation of mice and demonstrated enhanced suppressive activity of CD4+CD25+ cells in both *in vitro* and *in vivo* assays leading the authors to conclude that 1,25(OH)₂D₃ may be an important mediator by which UVB-irradiation exerts some of its immunomodulatory effects, although some notable differences were observed [103]. As discussed above, UVR irradiation influences the function of skin dendritic cells leading to changes in T cell homing, and enhancement of Treg cell frequency and functions. Exposure of murine antigen presenting cells to UVR reduces their antigen presentation capacity as shown through a UVB Treg transfer mouse model [104]. The induction of Tregs from the periphery to lung sites is vital for their inhibitory functions there. The chemokine receptor CCR4 is central for the recruitment of T cells into lung tissue, and absence of this homing marker was shown to impair the recruitment of Tregs into the lung accompanied by the development of inflammatory lung disease [105]. Whilst tissue-specific dendritic cells are able to reprogram the migration of UVR-induced Tregs [42], as far as we are aware this has not been investigated within the context of asthma or lung tissue homing.

In addition to studies in asthma, it may be possible to infer likely effects of skin UVR exposure on asthma through studies in other immunological conditions. In these UVR has been shown to impact upon numerous cellular and molecular pathways, leading to local and systemic immune modulation [10]. As reviewed in Norval *et al* there are additional photoreceptors in the skin that lead to the release of a number of immunomodulatory signalling molecules including IL-6, IL-10, platelet activating factor (PAF), prostaglandins, TNF α and PGE2 [10, 106], although the relevance of these findings to the airways and asthma is as yet unclear.

Vitamin D and asthma development

Asthma is often referred to as a developmental disease, in part due to its early onset. Many, but not all, observational studies suggest that low vitamin D intake during pregnancy is detrimentally associated with recurrent wheeze and the incidence of asthma in the offspring [107]; many more studies have addressed this with respect to protective effects on complications of pregnancy [108]. Two very recent studies addressing the impact on respiratory health of the infant have been published. Both administered daily regimes of vitamin D that were able to significantly increase vitamin D status of the mother, and demonstrated improvement in episodes of troublesome lung episodes ($p=0.02$) [109], and trends towards reduced recurrent respiratory wheeze and asthma ($p=0.051$), as well as significantly reduced allergic sensitizations, in the infants at age 3 years, even though asthma is generally not diagnosed until 5-6 years of age [110]. To the best of our knowledge no data exist on the impact of UVR/UVB exposures during pregnancy on asthma and respiratory related outcomes, such as recurrent wheeze, in the offspring.

Vitamin D is likely to impact both lung and immune maturation [111]. Early studies in rats born to mothers deprived of dietary vitamin D showed reduced lung compliance [112] whilst the offspring of vitamin D deficient mice had detrimentally altered lung function and structure [113]. In a human study, vitamin D pathway genes were shown to be over represented in developing normal human lung and a role for these developmental genes in asthma pathogenesis proposed [33]. Vasiliou *et al* reported that a vitamin D-deficient diet for female mice during the third trimester of pregnancy and lactation resulted in Th2 skewing, reduced IL-10, and eosinophilia in the offspring. It did not affect AHR, but

contributed to disease severity with worse eosinophilic inflammation and airway remodeling in house dust mite-induced airways disease in the offspring [114]. In contrast, Wittke *et al.* reported that mice lacking the VDR do not develop airway inflammation or experimental asthma [115, 116]. However, in the light of more recent reports it is plausible that these data may be explained by effects of vitamin D on lung development [33, 113] or even of vitamin D on immune cell signalling [117, 118]. The immunological effects of vitamin D at the maternal-foetal interface and in pregnancy have recently been reviewed elsewhere [119, 120], but are likely to demonstrate important differences to the effects extensively reported in adults, given that pregnancy and early life are a period of intense immune development.

Conclusion

The health of our airways requires the efficient clearance of pathogens in the face of well-controlled inflammatory responses in order to minimise damage to the fragile airway structure with the capacity to impair the primary airway function of gaseous exchange. Most studies of the impact of UVR on immune function suggest that these are mediated within the UVB range, and focus predominantly on effects in the skin. These show that UVB, at physiological and modest doses, suppresses adaptive T cell immunity through effects on Langerhan's cells, leading to the induction of antigen-specific regulatory T cells, and acts on innate immune cells to promote the synthesis of antimicrobial peptides that are protective against bacterial and viral infections. This bears many similarities with the major effects of vitamin D on structural cells and cells of the innate and adaptive immune response, which have been proposed to explain the described associations between low vitamin D status with impaired airway function and poor asthma control. In addition vitamin D has well-described effects to enhance responsiveness to steroids. Clinical trials to assess the impact of vitamin D on lung function, respiratory tract infections and asthma are being performed in pregnancy, pediatric and adult cohorts (see clinicaltrials.gov); these studies overall suggest positive effects. Although these effects are frequently modest, within a disease with such high prevalence as asthma (estimates of greater than 300 million worldwide), this could translate into major public health benefit. These studies with vitamin D beg the issue

of whether safe, modest and physiological levels of UVB irradiation will afford similar protection.

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