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Immunoregulatory mechanisms of vitamin D relevant to respiratory health and asthma

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Vitamin D deficiency is prevalent among people with various immune-mediated conditions, including autoimmune diseases and asthma. Serum 25(OH)D levels inversely correlate with asthma severity, glucocorticoid responsiveness/dosage, and markers of pathogenesis, such as airway remodeling, IgE, and eosinophilia. Trials involving supplementation with active vitamin D or a precursor are beginning to emerge with variable results that, in part, reflect differences in study design. This review looks at the mechanisms by which vitamin D may protect against asthma, including increasing glucocorticoid responsiveness, skewing immune cells towards a regulatory phenotype, reducing the incidence of infections, airway remodeling, eosinophilia, and lowering the levels of IgE. Also discussed is the therapeutic potential for vitamin D, which is likely to be applicable to immune-mediated conditions beyond simply asthma.

Keywords: vitamin D; asthma; immunoregulatory; therapeutic

Asthma

Asthma is a disease characterized by reversible bronchial constriction, mucus hyperplasia, and airway hyperresponsiveness (AHR).¹ Asthma prevalence continues to increase and despite a large amount of research in the field identifying some mechanisms underlying asthma development, large holes in our knowledge still exist.² In the majority of people with asthma, symptoms are well controlled with β_2 -adrenergic agonists and glucocorticoids; however, such therapies are ineffective at controlling the disease in some patients. Although refractory symptoms and exacerbations despite high-dose inhaled corticosteroids can be attributed to poor inhaler technique, lack of compliance, comorbidities, and incorrect diagnosis, there is a cohort of asthmatics for whom glucocorticoid treatment is clinically ineffective (steroid refractory/resistant).³ These people suffer considerable morbidity and are expensive to manage, highlighting a need to improve our understanding of the disease and how to treat it.

There is a growing consensus that asthma is a disease of different endotypes that result from different pathogenic mechanisms in different patients and that need different therapies.⁴ Therefore, to facilitate progress, the heterogeneity of asthma and its various endotypes, for example, eosinophilic, T helper (T_H)2-mediated, and neutrophilic asthma, must be clearly defined and taken into consideration.^{1,4}

Vitamin D

Vitamin D can be ingested through dietary substances, such as oily fish or supplements, but it is predominantly synthesized by ultraviolet B (UVB) radiation from sunlight that induces photolysis of skin resident 7-dehydrocholesterol into vitamin D_3 . CYP27A1 in the liver subsequently hydroxylates this metabolite into 25-hydroxyvitamin D_3 (25(OH) D_3), which is converted to the active metabolite (1 α ,25-dihydroxyvitamin D_3 (1,25(OH) $_2D_3$) by the mitochondrial enzyme CYP27B1.⁵ This enzyme is expressed in the kidney but also in various peripheral cells, including epithelial cells, T cells, macrophages,

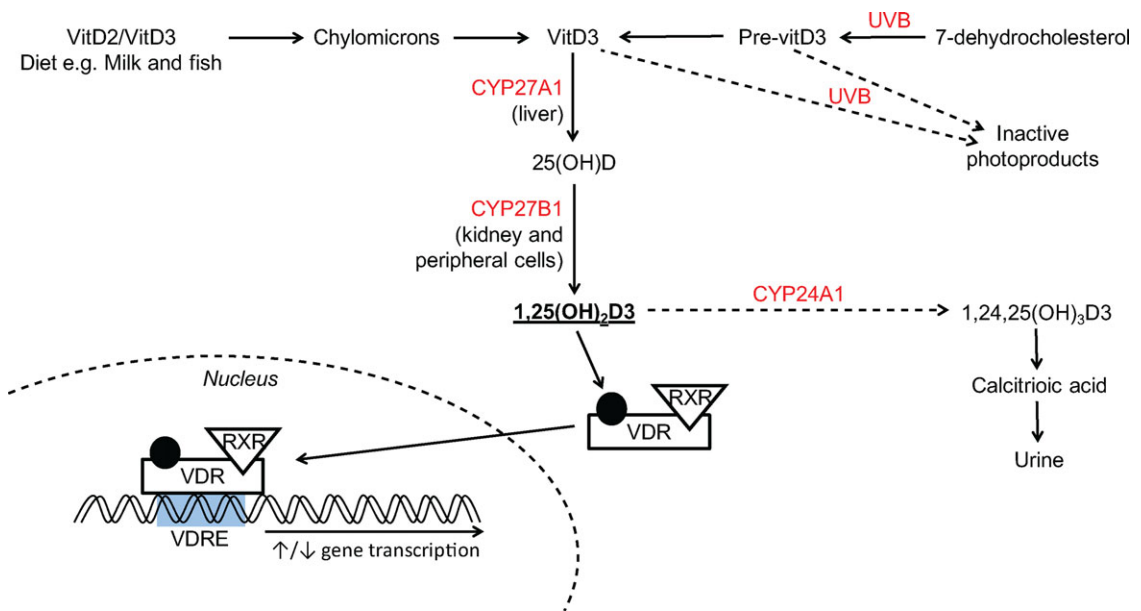


Figure 1. 1,25(OH)₂D₃ metabolism and receptor binding. Vitamin D₃ (VitD₃) is obtained from dietary sources or metabolized by ultraviolet B (UVB) irradiation of 7-dehydrocholesterol. VitD₃ is then converted to its major form in the body, 25(OH)D, by CYP27A1 in the liver. Active vitamin D (1,25(OH)₂D₃) is synthesized locally by the action of CYP27B1 in the kidney, although emerging evidence reveals that conversion also occurs locally in tissues, which is likely to be important for immunomodulation. 1,25(OH)₂D₃ then engages the vitamin D receptor (VDR) and members of the retinoic X receptor (RXR) family. This complex translocates to the nucleus, where it binds the vitamin D response element (VDRE) in the promoter of genes to alter their transcription. Rapid inactivation of vitamin D by UVB irradiation and by CYP24A1 helps to control its activity.

and dendritic cells (DCs), which are reported to produce 1×10^{-9} to 6×10^{-8} M 1,25(OH)₂D₃ from an excess of 25(OH)D in culture.^{5–10} 1,25(OH)₂D₃ binds to the nuclear hormone vitamin D receptor (VDR), then heterodimerizes with receptors of the retinoic X receptor family to translocate into the nucleus. Similarly to CYP27B1, VDR is widely expressed by cells of the immune system, enabling them to respond to 1,25(OH)₂D₃.⁵ In the nucleus, 1,25(OH)₂D₃ ligated to VDR binds to the vitamin D response element (VDRE) in the promoter of target genes to influence gene transcription. The duration of activity of 1,25(OH)₂D₃ is limited by being rapidly catabolized by CYP24A1 (Fig. 1).¹¹

25(OH)D is the major form of vitamin D in the body and therefore the circulating serum concentration of this metabolite is a widely accepted measure of an individual's vitamin D status. There is, however, disagreement as to what is a sufficient level.¹² Generally, serum levels of 25(OH)D of less than 20 ng/mL (50 nmol/L) are classified as a deficiency, with 20–30 ng/mL (50–75 nmol/L) indicating an insufficiency.¹³ On the basis of these definitions, 20–80% of community-dwelling individuals from

the United States, Canada, and Europe are vitamin D deficient, with nonwhite people being at a particularly high risk due to reduced cutaneous production of vitamin D₃.¹³

Vitamin D and asthma: correlations and epidemiology

Although traditionally known for its effects on bone and mineral homeostasis, vitamin D is increasingly recognized as a complex immunomodulatory molecule. As such, the role of vitamin D in immune-mediated diseases, including asthma, has come under the spotlight.

The importance of vitamin D for pulmonary health was initially highlighted when a positive correlation between serum 25(OH)D and lung function was identified by Black *et al.*,¹⁴ an observation that has since been made repeatedly.^{15–25} This relationship has been extended in the context of asthma, revealing a greater incidence of vitamin D insufficiency in pediatric asthmatic patients compared with healthy controls.^{17,26} Similarly, many, but not all, longitudinal studies conclude that lower intake of vitamin D during pregnancy is

associated with an elevated risk of wheeze/asthma during childhood,^{27–31} an effect that was significant in a meta-analysis.³⁰

Possibly more so than the incidence of asthma, vitamin D insufficiency seems to play an important role in determining asthma severity and therapeutic control. For example, serum 25(OH)D negatively correlates with the severity of asthma (e.g., number of exacerbations) in pediatric patients^{17,24} and a requirement for higher-dose glucocorticoids.^{25,32,33} Inverse correlations have also been documented between serum 25(OH)D and characteristics of asthma, such as airway remodeling, IgE, and eosinophil levels, as well as AHR^{17,25,33} (Table 1).

A major cause of asthma exacerbations is respiratory tract infections (RTIs),³⁴ and therefore anything that can reduce their incidence is likely to improve asthma control. In this context, serum 25(OH)D inversely correlates with the incidence of acute RTIs in multiple cross-sectional, case-control, and cohort studies.^{35,36} Out of the 25 association studies recently reviewed by Jolliffe *et al.*,³⁵ only two suggested that vitamin D increased the incidence of RTIs, and five reported no significant effect.

Genetic studies also point to a role for vitamin D in asthma and atopy. There are a number of VDR polymorphisms associated with increased risk of asthma, and many asthma-associated genes are known to be vitamin D regulated.^{37–40} One study reported that multiple VDR polymorphisms were associated with IgE levels in a gender-specific manner.³⁷ Such findings indicate that genotyping patients may help to identify asthma endotypes and the susceptibility of individuals to the consequences of vitamin D deficiency.

Early vitamin D intervention clinical studies

The few clinical trials that have been conducted suggest a role for vitamin D in improving lung function, reducing asthma exacerbations, disease severity, and/or reducing glucocorticoid dependence.^{41–44} For example, Urashima *et al.*⁴¹ showed diminished exacerbations in children supplemented with 1200 IU vitamin D₃ for 4 months as a secondary outcome. This may, in part, be a result of the lower incidence of influenza A infection, which was observed as the primary outcome in children given the vitamin D supplement.

A minority of studies, however, do show a lack of, or even adverse, effects of vitamin D on asthma at a correlative level^{38,45–48} and as an outcome of clinical trials.^{49,50} Accurately interpreting the data from such studies is a considerable challenge due to the low numbers of participants in each trial and considerable variation in study design. The choice of primary outcome measure and patient group also differs between studies, which may affect whether vitamin D is beneficial. In addition, the dosing regimen and duration of vitamin D supplementation, inclusion criteria, baseline vitamin D status, and controls are very heterogeneous between studies. One cannot yet conclude whether a lack of effect in some trials is due to insufficient length or dose of supplementation, the fact that participants were already vitamin D sufficient, or to a genuine lack of effect in some asthma populations. Furthermore, vitamin D therapies might only be effective in vitamin D–insufficient individuals and/or of particular benefit to patients with specific disease endotypes. Although one can speculate, it is still early and we need more well-designed clinical trials on which to base our judgment.

Observational and early clinical intervention studies clearly suggest that vitamin D may modulate the incidence and progression of asthma. The rest of this review therefore explores the mechanisms by which vitamin D may be protective in asthma and the vitamin's therapeutic potential, with reference to particular aspects of asthma pathology and disease endotypes.

Mechanisms by which vitamin D protects against asthma

T_H2-mediated allergic asthma

The major endotype of asthma is a T_H2-mediated pathology wherein IL-4, IL-5, and IL-13 initiate and maintain the disease phenotype.⁵¹ Each cytokine has unique functions, with IL-4 promoting IgE production, IL-5 facilitating eosinophil survival, and IL-13 altering the actions of resident immune and structural cells. IgE plays a key role in acute asthma exacerbations and the propagation of airway inflammation, particularly in atopic individuals.⁴ Vitamin D can both inhibit or enhance T_H2 responses in different experimental systems (reviewed in Ref. 52; Fig. 2), which is a potential concern in allergy and asthma. Notably, serum 25(OH)D negatively correlates with total and aeroallergen-specific IgE in

Table 1. Correlations between serum 25(OH)D and indicators of asthma incidence, severity, and pathogenesis

	Effect of higher serum 25(OH)D in asthmatics		
	Beneficial	None	Adverse
Predictor of wheeze/asthma (maternal or individual's serum 25(OH)D)	Freishtat <i>et al.</i> ¹¹⁹ Camargo <i>et al.</i> ³¹ Gupta <i>et al.</i> ¹⁷ Chinellato <i>et al.</i> ²¹ Keet <i>et al.</i> ¹²⁰ Hollams <i>et al.</i> ¹²¹ Alyasin <i>et al.</i> ¹²² Bener <i>et al.</i> ²⁶ Maalmi <i>et al.</i> ⁶⁵	Devereux <i>et al.</i> ¹²³ Hughes <i>et al.</i> ⁴⁷ Morales <i>et al.</i> ¹²⁴ Menon <i>et al.</i> ¹²⁵ Mai <i>et al.</i> ¹²⁶ Pike <i>et al.</i> ¹²⁷ Gergen <i>et al.</i> ⁴⁸ Ozaydin <i>et al.</i> ¹²⁸	Gale <i>et al.</i> ⁴⁵ Goleva <i>et al.</i> ³³ Tolppanen <i>et al.</i> ¹²⁹
Disease severity (e.g., lung function, hospitalizations, and exacerbations)	Black <i>et al.</i> ¹⁴ Brehm <i>et al.</i> ⁵⁶ Brehm <i>et al.</i> ¹³⁰ Sutherland <i>et al.</i> ¹⁵ Searing <i>et al.</i> ¹⁶ Li <i>et al.</i> ¹⁹ Gupta <i>et al.</i> ⁵⁶ Majak <i>et al.</i> ⁴² Chinellato <i>et al.</i> ²⁰ Chinellato <i>et al.</i> ²¹ Tolppanen <i>et al.</i> ²² Alyasin <i>et al.</i> ¹²² Lewis <i>et al.</i> ⁴⁹ Wu <i>et al.</i> ²³ Majak <i>et al.</i> ³² Brehm <i>et al.</i> ²⁴ Maalmi <i>et al.</i> ⁶⁵ Korn <i>et al.</i> ²⁵ Montero-Arias <i>et al.</i> ¹³¹	Shaheen <i>et al.</i> ¹⁸ Menon <i>et al.</i> ¹²⁵ Pike <i>et al.</i> ¹²⁷ Gergen <i>et al.</i> ⁴⁸ Tolppanen <i>et al.</i> ¹²⁹ Krobtrakulchai <i>et al.</i> ¹³²	Gergen <i>et al.</i> ⁴⁸
Glucocorticoid responsiveness	Brehm <i>et al.</i> ⁵⁶ Brehm <i>et al.</i> ¹³⁰ Sutherland <i>et al.</i> ¹⁵ Searing <i>et al.</i> ¹⁶ Gupta <i>et al.</i> ¹⁷ Majak <i>et al.</i> ³² Goleva <i>et al.</i> ³³ Korn <i>et al.</i> ²⁵	Alyasin <i>et al.</i> ¹²² Gergen <i>et al.</i> ⁴⁸ Montero-Arias <i>et al.</i> ¹³¹ Krobtrakulchai <i>et al.</i> ¹³²	
IgE	Brehm <i>et al.</i> ⁵⁶ Searing <i>et al.</i> ¹⁶ Gupta <i>et al.</i> ¹⁷ Hollams <i>et al.</i> ¹²¹ Sharief <i>et al.</i> ⁵³ Goleva <i>et al.</i> ³³ Gergen <i>et al.</i> ⁴⁸	Brehm <i>et al.</i> ¹³⁰ Li <i>et al.</i> ¹⁹ Chinellato <i>et al.</i> ²¹ Brehm <i>et al.</i> ²⁴ Korn <i>et al.</i> ²⁵ Montero-Arias <i>et al.</i> ¹³¹	Hypponen <i>et al.</i> ¹³³ — both low and high extremes of serum 25(OH)D associated with elevated IgE
Eosinophilia	Brehm <i>et al.</i> ⁵⁶ Hollams <i>et al.</i> ¹²¹ Korn <i>et al.</i> ²⁵	Brehm <i>et al.</i> ¹³⁰ Searing <i>et al.</i> ¹⁶ Li <i>et al.</i> ¹⁹ Gupta <i>et al.</i> ¹⁷ Alyasin <i>et al.</i> ¹²² Chinellato <i>et al.</i> ²¹ Montero-Arias <i>et al.</i> ¹³¹ Krobtrakulchai <i>et al.</i> ¹³²	
T _{regs} cells	Maalmi <i>et al.</i> ⁶⁵ Majak <i>et al.</i> ³² Chambers <i>et al.</i> ⁶⁶		

Continued

Table 1. Continued

	Effect of higher serum 25(OH)D in asthmatics		
	Beneficial	None	Adverse
Respiratory tract infections	Wayse <i>et al.</i> ¹³⁴ Laaksi <i>et al.</i> ¹³⁵ Ginde <i>et al.</i> ¹³⁶ Roth <i>et al.</i> ¹³⁷ Jartti <i>et al.</i> ¹³⁸ Sabetta <i>et al.</i> ¹³⁹ Freishtat <i>et al.</i> ¹¹⁹ Berry <i>et al.</i> ¹⁴⁰ Camargo <i>et al.</i> ³¹ Morales <i>et al.</i> ¹²⁴ Hirani <i>et al.</i> ¹⁴¹ Science <i>et al.</i> ¹⁴² Aregbesola <i>et al.</i> ¹⁴³	Gale <i>et al.</i> ⁴⁵ McNally <i>et al.</i> ¹⁴⁴ Porojnicu <i>et al.</i> ¹⁴⁵	

patients,^{17,53} suggesting that compensatory mechanisms exist to counter any effects of vitamin D enhancement of T_H2 responses. For example, increased IgE-mediated sensitization to common allergens, such as peanut, ragweed, and oak, has been observed in vitamin D–deficient children.⁵³ *In vitro* 1,25(OH)₂D₃ reduces the production of IgE from peripheral human B cells and increases expression of IL-10, skewing the cells towards a more regulatory phenotype.^{54,55} Further work is certainly needed to clarify this controversy.

Another characteristic of T_H2-mediated pathology in asthma is eosinophilia wherein eosinophils secrete proinflammatory cytokines, chemokines, leukotrienes, and matrix metalloproteinases (MMPs). Since these mediators are thought to contribute to asthma, it is therefore of interest that sputum eosinophil counts are inversely correlated with serum 25(OH)D.^{25,56,57} Furthermore, topical application of 1,25(OH)₂D₃ on OVA-sensitized mice reduced BALF eosinophil counts upon OVA challenge.⁵⁸ It is proposed that by upregulating expression of CXCR4 on eosinophils, 1,25(OH)₂D₃ may recruit eosinophils away from sites of inflammation.⁵⁹

T regulatory cells

T regulatory lymphocytes (T_{reg} cells) play a key role in suppressing excessive immune responses but are reported to be diminished in proportion and suppressive activity in asthmatic individuals.⁶⁰ Adoptively transferring CD4⁺ CD25⁺ T cells into a mouse model of airway inflammation resulted in suppressed AHR, T_H2 responses, and eosinophilia.⁶¹ A subsequent study similarly showed reduced AHR in

a rat model of asthma following induction of CD4⁺ CD25⁺ Foxp3⁺ LAG3⁺ CTLA⁺ CD45RC⁺ T cells.⁶² It is therefore beneficial that CD4⁺ CD25⁺ T_{reg} cells were upregulated in mice treated with either topical 1,25(OH)₂D₃ or UVB radiation and were more suppressive than cells from control mice *in vitro*, *in vivo*, and following adoptive transfer.^{63,64} In humans, a positive correlation exists between serum 25(OH)D and FoxP3 expression,^{32,65,66} and treatment of CD4⁺ T cells with 1,25(OH)₂D₃ increases the expression of FoxP3 and the inhibitory costimulatory molecules CTLA-4^{9,67–69} and PD1.⁶⁸

Levels of anti-inflammatory IL-10 are diminished in the bronchial lavage of severe therapy-resistant pediatric asthmatics with lower production by their peripheral blood mononucleated cells.⁷⁰ However, serum 25(OH)D positively correlates with levels of IL-10.^{71,72} In addition, 1,25(OH)₂D₃ upregulates expression of IL-10 at the gene and protein level in various experimental settings in mice,^{73–75} as well as in human CD4⁺ T cell cultures.^{68,76} *In vitro* enhancement of IL-10 is seen optimally after 7–14 days,⁶⁸ a relatively slow effect compared to *ex vivo* data wherein an increase in IL-10 mRNA is observed 24–72 h after administration of oral 1,25(OH)₂D₃.⁷⁶ The time difference suggests that vitamin D may be acting as a cofactor to promote IL-10 secretion.

CD39 is an ectonucleotidase that catalyzes the dephosphorylation of adenosine triphosphate and adenosine diphosphate into adenosine monophosphate, which is subsequently converted into immunoregulatory adenosine by CD73.⁷⁷ Although this molecule is downregulated in severe asthmatics, the addition of 1,25(OH)₂D₃ to CD4⁺ T cell

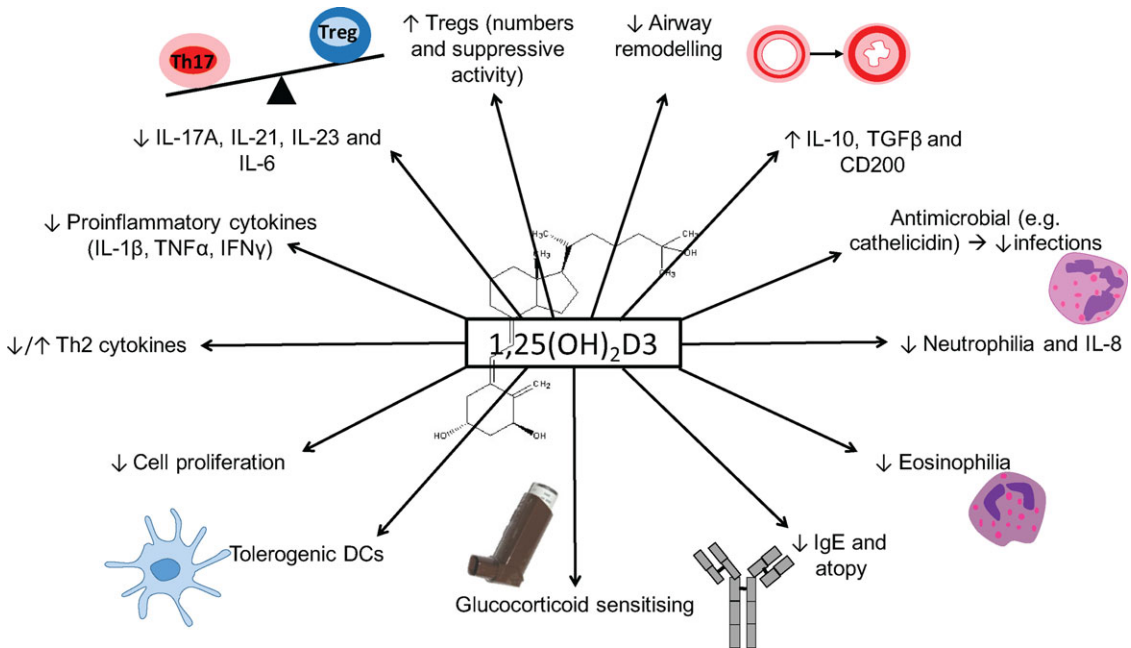


Figure 2. Mechanisms by which $1,25(\text{OH})_2\text{D}_3$ protects against asthma.

cultures increases CD39 expression.⁷⁸ This is of particular interest because Fletcher *et al.*⁷⁹ showed an important role for CD39 in suppressing $\text{T}_\text{H}17$ responses, which, as discussed below, are heightened in severe asthmatics.

One way that vitamin D promotes T_reg cell populations is by impairing the maturation of DCs. This can be seen by reduced surface expression of CD40, CD80, CD86, HLA-DR, and the maturation marker CD83, on $1,25(\text{OH})_2\text{D}_3$ -treated DCs.^{80,81} $1,25(\text{OH})_2\text{D}_3$ is also reported to promote production of CCL22, a T_reg cell chemoattractant, from myeloid DCs.⁸² In addition, $1,25(\text{OH})_2\text{D}_3$ induces the expression of the inhibitory receptor immunoglobulin-like transcript 3 and programmed death ligand 1 on the surface of DCs, both of which are molecules that impair the function of T effector cells while promoting T_reg cell generation.^{67,83}

Severe neutrophilic asthma

Many patients with severe asthma and a resistance to conventional therapies have a neutrophilic endotype that is associated with elevated levels of IL-17A, according to both mouse models^{84,85} and human studies.^{78,86–88} Although IL-17A plays a critical role in defense against bacterial and fungal mucosal

infections, an excess is implicated in asthma and other immune-mediated diseases. IL-17A is thought to negatively affect asthma by promoting AHR, neutrophilia, airway remodeling, and production of proinflammatory cytokines.^{84,85,89} This is intriguing, considering that dexamethasone can upregulate production of IL-17A from cells *in vitro*.⁷⁸ It may therefore be that glucocorticoids are not only ineffective in steroid refractory asthmatics, but may actually contribute to disease progression by heightening the $\text{T}_\text{H}17$ response. Possible other mechanisms for unresponsiveness of immune cells to glucocorticoids have been reviewed elsewhere⁹⁰ but include increased expression of the nonligand-binding glucocorticoid receptor- β isoform, altered histone acetylation, and/or vitamin D deficiency.

Mouse studies reveal that $1,25(\text{OH})_2\text{D}_3$ diminishes production of IL-17A via modulation of the VDR pathway,^{74,75,91} a finding that is consistent in humans.^{9,78,92,93} This observation was recently extended to show that $1,25(\text{OH})_2\text{D}_3$ can overcome the induction of IL-17A by dexamethasone.^{70,78} IL-22 is another $\text{T}_\text{H}17$ -associated cytokine that is implicated in asthma and is downregulated by $1,25(\text{OH})_2\text{D}_3$.^{75,78} However, in some studies IL-22 is reported to suppress lung inflammation, highlighting the complexity of immune networks, such

that the stage of disease, type of cell studied, and the location, all affect functionality.⁹⁴

Transcriptome analysis of 1,25(OH)₂D₃-treated mouse CD4⁺ T cells showed that in addition to IL-17A and IL-22, mRNA expression of T_H17-associated genes IL-23R, IL-17E, and the master transcription factor ROR γ t were reduced, although expression of IL-21 and the aryl hydrocarbon receptor were unaltered.⁷⁵ The presence of IL-23 is thought to be important for T_H17 cells to mediate their pathogenic effects.⁹⁵ As such, it is of interest that 1,25(OH)₂D₃ downregulates IL-23 at the RNA and protein level, which was concurrently associated with diminished gut inflammation in mice.⁹⁶ One mechanism by which 1,25(OH)₂D₃ may reduce the number of T_H17 cells is by limiting expression of transcription factors RORc, RUNX1, and NFAT, as well as the cytokine IL-6, all of which promote the differentiation and maintenance of T_H17 cells.^{74,97}

IL-8 is a key neutrophil chemoattractant and, as such, is thought to negatively affect asthma, particularly severe asthma, by facilitating neutrophilia.^{97,98} However, 1,25(OH)₂D₃ appears to be capable of dampening levels of IL-8 because daily supplementation of colorectal patients with 800 IU vitamin D₃ for 6 months significantly reduced expression.⁹⁹

Glucocorticoid sensitizing

In addition to the *in vivo* correlative clinical data illustrating a glucocorticoid-sensitizing effect of vitamin D (Table 1), evidence exists *in vitro*.^{100,101} Xystrakis *et al.*¹⁰¹ showed that peripheral blood CD4⁺ T cells from steroid refractory patients were unable to increase secretion of anti-inflammatory IL-10 *in vitro* when treated with dexamethasone, whereas cells from steroid-sensitive individuals and healthy controls were able to do so. However, adding 1,25(OH)₂D₃ in culture or supplementing steroid refractory asthmatics with 0.5 μ g 1,25(OH)₂D₃ per day for 7 days restored the dexamethasone induction of IL-10.¹⁰¹ A proposed mechanism for this is that 1,25(OH)₂D₃ can overcome downregulation of the glucocorticoid receptor by dexamethasone.

Vitamin D and respiratory tract infections

Since viral and bacterial infections are a major cause of asthma exacerbations,³⁴ one can extrapolate that antimicrobial actions of vitamin D will be beneficial in the disease setting. In mice^{58,102} and certain human cells, including epithelial cells, keratinocytes, and monocytes,^{103–105} 1,25(OH)₂D₃ treatment in-

creases levels of antimicrobial peptides, such as cathelicidin, and has been reported to concurrently reduce the incidence of infection.¹⁰⁶ 1,25(OH)₂D₃ reduces induction in airway epithelial cells of proinflammatory chemokines and cytokines by respiratory syncytial virus infection, without compromising control of viral replication;¹⁰⁵ this may be an important mechanism by which vitamin D helps to control viral-induced asthma exacerbations without necessarily reducing the incidence of infections. 1,25(OH)₂D₃ also promotes expression of CD200, a molecule that suppresses the proinflammatory activity of local innate immune cells, in peripheral and nasal airway human T cells.¹⁰⁷

Airway remodeling

Airway remodeling is a complex phenomenon that occurs in asthma to irreversibly reduce lung function and is generally not well controlled with current therapies.¹⁰⁸ In addition to an increased number of fibroblasts plus vascular and epithelial cell changes, increased airway smooth muscle (ASM) mass is a key contributing factor to airway remodeling. In mouse models of asthma, increasing levels of vitamin D reduced ASM mass, subepithelial deposition, and goblet cell hyperplasia.^{109,110} Correspondingly, serum 25(OH)D inversely correlates with ASM mass in pediatric asthmatics¹⁷ and addition of 1,25(OH)₂D₃ to human ASM *in vitro* reduces cell proliferation.^{111,112} Vitamin D may also be beneficial in asthma by reducing expression of ADAM33 and MMP9 on ASM, both of which are enzymes that are implicated in airway remodeling.^{111,113} Furthermore, the production of proinflammatory cytokines, including IL-6 and IL-8, from ASM and extracellular matrix proteins, such as collagen and fibronectin from fibroblasts, is impaired by 1,25(OH)₂D₃.^{114,115}

Therapeutic implications of vitamin D sufficiency in respiratory health

The definitions of vitamin D insufficiency/deficiency are currently based on the levels required for vitamin D to mediate its effects on bone and mineral homeostasis; this does not, however, take into consideration the required dose for any immunomodulatory actions. Complexity is further added by the fact that the body has considerable capacity to compensate for low levels of vitamin D intake via mechanisms such as those mediated by parathyroid hormone, resulting in a nonlinear

relationship between vitamin D intake and its biological effects.¹¹⁶

Autier *et al.*¹¹⁷ recently published a systematic review in which they concluded that an inverse relationship exists between serum 25(OH)D and multiple markers of poor health, but that vitamin D supplementation had no beneficial effect in a meta-analysis of multiple outcomes relevant to many different diseases. There is, however, limited reference to respiratory measures in this article, and variable end points are pooled in the study, which we believe may obscure positive effects of vitamin D on specific outcomes. To facilitate understanding of the relationship between vitamin D and respiratory health, as well as reach a consensus on the required dose, longitudinal clinical trials with large well-defined cohorts of asthmatics will be extremely useful.

To date, no biologic has successfully helped to control asthma unless used in a specific subgroup of phenotyped patients that are selected on the basis of scientific reasoning, and it seems likely that the same applies to vitamin D. Clinical trials need to be designed on the basis of our mechanistic knowledge of the pathways regulated by vitamin D. For example, given the *in vitro* evidence for vitamin D increasing glucocorticoid sensitivity and inhibiting eosinophilia, a suitable primary outcome for a vitamin D intervention in an eosinophilic asthma trial might be whether vitamin D is able to reduce the dose of inhaled corticosteroids required to maintain asthma control without development of a sputum eosinophilia. Alternative outcomes of interest would be a reduction in exacerbations provoked by RTIs in asthmatic individuals, or a reduction in sputum neutrophilia in severe asthmatics.

Vitamin D is a relatively safe and cheap treatment with great therapeutic potential. Current work proposes that vitamin D itself should be administered as an adjuvant to help reduce mediators involved in asthma pathogenesis, rather than as a replacement for current therapies. For example, vitamin D can increase glucocorticoid responsiveness and reduce the dose of glucocorticoids required for asthma control, therefore limiting glucocorticoid side effects. In addition to determining required levels of vitamin D, work is needed to establish how best to administer it. In order to avoid fluctuations and sustain a healthy level, daily supplementation is likely to be preferable to bolus doses. However, care must be taken to ensure that supraphysiological levels of vi-

tamin D are not induced because extreme variations, as well as very high levels, are associated with impaired immune responses and hypercalcaemia.¹¹⁸ It may be that vitamin D analogues are able to mediate the beneficial effects of vitamin D without the risk of potential side effects.

Conclusion

Multiple epidemiological studies show associations between vitamin D deficiency and various immune-mediated diseases, including autoimmunity and asthma. Therefore, the ability of vitamin D to increase several immunoregulatory pathways is very relevant to diseases beyond simply asthma. In addition to improving clinical responsiveness to glucocorticoids, vitamin D may offer potential to patients for improved tolerizing regimens, such as allergen immunotherapy or even adoptive cell transfers.

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Conflicts of interest

The authors declare no conflicts of interest.

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