

# The role of vitamin D in asthma

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**Objective:** To review the current literature on vitamin D and asthma, discussing the possible roles of vitamin D on asthma pathogenesis and the potential consequences of vitamin D deficiency.

**Data Sources:** PubMed database was searched from 1950 to 2009. Keywords used included *asthma, vitamin D, inflammation, airway smooth muscle and cytokines*.

**Study Selection:** Articles were selected based on relevance to the subject.

**Results:** Vitamin D deficiency has been associated with epidemiologic patterns observed in the asthma epidemic. Vitamin D deficiency is more common with obesity, African American ethnicity, and westernization of countries with higher-risk populations for asthma. Evidence suggests that vitamin D deficiency is associated with increased airway hyperresponsiveness, lower pulmonary functions, worse asthma control, and possibly steroid resistance. Lung epithelial cells express high baseline levels of 1 $\alpha$ -hydroxylase. This allows the conversion of inactive calcidiol to active calcitriol locally within the lung. Calcitriol has been shown to inhibit the synthesis and release of certain cytokines, such as RANTES, platelet-derived growth factor, and matrix metalloproteinases, from bronchial smooth muscle cells, thereby leading to decreased lung inflammation and smooth muscle cell proliferation. Vitamin D also increases synthesis of interleukin 10 by CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> T-regulatory cells and dendritic cells, while concurrently inhibiting dendritic cell activation by downregulating expression of costimulatory molecules CD40 and CD80/86. Vitamin D is also capable of inducing the expression of several anti-infective molecules, such as cathelicidin. Thus, vitamin D has a number of biologic effects that are likely important in regulating key mechanisms in asthma.

**Conclusions:** We hypothesize that vitamin D supplementation may lead to improved asthma control by inhibiting the influx of inflammatory cytokines in the lung and increasing the secretion of interleukin 10 by T-regulatory cells and dendritic cells.

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**Off-label disclosure:** Drs Sandhu and Casale have indicated that this article does not include the discussion of unapproved/investigative use of a commercial product/device.

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## INTRODUCTION

Asthma is a considerable public health issue, with more than 300 million people affected worldwide. It remains the most common chronic disease of childhood in the world. In most cases, asthma has its onset in early childhood, with 80% to

90% of cases diagnosed in patients before the age of 6 years. The prevalence of asthma is greatest in westernized, industrialized nations, with a predilection toward countries farther away from the equator.<sup>1</sup> In the United States, the lifetime prevalence of asthma is approximately 11.6 per 100 persons. The most recent data from 2006 indicate that close to 23 million Americans have asthma. More than 50% of these individuals had an asthma attack in 2006. In addition to the high morbidity of asthma, approximately 4,000 lives are lost per year. The economic cost of asthma is close to \$20 billion annually. Asthma ranked in the top 10 prevalent conditions causing limitations of activity.<sup>2</sup> Recent studies indicate that current pharmacotherapy is often inadequate in effectively

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treating patients with asthma. In the Gaining Optimal Asthma Control study, only approximately 50% of patients had well-controlled asthma despite recommended treatment with an inhaled corticosteroid and a long-acting  $\beta$ -agonist.<sup>3</sup> These data suggest a need for efforts to identify causes of the asthma epidemic and treatments aimed at the identified risk factors.

Evidence suggests there may be a cause-and-effect relationship with vitamin D deficiency and the increased incidence of asthma because vitamin D deficiency is associated with increased airway hyperresponsiveness, lower pulmonary functions, worse asthma control, and possibly steroid resistance. Indeed, vitamin D deficiency has been associated with epidemiologic patterns observed in the asthma epidemic. For example, vitamin D deficiency is more common with obesity, African American ethnicity, and westernization of countries reflecting higher-risk populations for asthma.

A limited number of studies have looked at pulmonary function parameters of asthma and chronic obstructive pulmonary disease and their relationship to vitamin D. In the late 1970s, Utz et al<sup>4</sup> published a small randomized, double-blind, placebo-controlled study looking at the effects of oral calcium and vitamin D supplementation on pulmonary function. They found that compared with the placebo group, the active treatment group had statistically significant increases in forced expiratory volume in 1 second (FEV<sub>1</sub>). Black and colleagues<sup>5</sup> examined the relationship between serum vitamin D levels and lung function in a cross-sectional analysis of data from the Third National Health, Nutrition and Examination Survey (NHANES). This survey was conducted from 1988 to 1994 and included 14,091 people who had undergone spirometry and measurements of serum 25-hydroxy vitamin D (25[OH]D<sub>3</sub>) levels. The serum 25(OH)D<sub>3</sub> concentration was inversely related to body mass index. It was lower in non-Hispanic blacks and Mexican Americans and participants who smoked more than 20 cigarettes per day. They examined the relationship between percentage of predicted FEV<sub>1</sub> and forced vital capacity (FVC) values and the circulating concentration of 25(OH)D<sub>3</sub>. After adjustment for confounders, the mean FEV<sub>1</sub> was 126 mL (SE, 22 mL) and the mean FVC was 172 mL (SE, 26 mL) greater for the highest quintile of serum 25(OH)D<sub>3</sub> level (>85.7 nmol/L) compared with the lowest quintile (<40.4 nmol/L;  $P < .0001$ ). Thus, it

appears as though there is a relationship between serum concentrations of 25(OH)D<sub>3</sub> and FEV<sub>1</sub> and FVC.

In a recent study published in the *American Journal of Respiratory and Critical Care Medicine*, 28% of children living in Costa Rica had vitamin D deficiency.<sup>2</sup> This was an interesting observation given the prevalence of sunlight; however, darker skin pigmentation contributes to low cutaneous production of vitamin D. These investigators also examined the relationship between serum 25(OH)D<sub>3</sub> levels and asthma and allergic diseases. They found that higher serum 25(OH)D<sub>3</sub> levels were significantly and inversely associated with total IgE levels and peripheral blood eosinophil counts. A higher serum 25(OH)D<sub>3</sub> level was also associated with reduced odds of any hospitalization in the previous year, lower use of anti-inflammatory medications for asthma, and less airway hyperresponsiveness. A 10-ng/mL increase in serum 25(OH)D<sub>3</sub> was associated with a decrease of 25 IU/mL in total IgE and of 29/mm<sup>3</sup> in eosinophils in peripheral blood. Higher serum 25(OH)D<sub>3</sub> levels were also associated with reductions in antigen specific IgE and skin test size.

An important question is what mechanism does vitamin D use to exert its biologic effects on airway inflammation, remodeling and hyperresponsiveness, and thereby asthma control. The answer to this question will invariably be complicated given the wide-ranging effects of vitamin D on airway epithelium, bronchial smooth muscle, and immune cells central to the pathogenesis of asthma (Table 1). This article reviews the current literature on vitamin D and asthma, discussing the possible roles of vitamin D on asthma pathogenesis and the potential consequences of vitamin D deficiency. We searched PubMed from 1950 to 2009 with keywords *asthma, vitamin D, inflammation, airway smooth muscle, cytokines* and selected articles based on relevance to the subject.

## VITAMIN D OVERVIEW

The first step in the biosynthesis of vitamin D involves UV light from the sun. Seven-dehydrocholesterol is distributed in the skin. After exposure to the UV-B spectrum, 7-dehydrocholesterol is converted to previtamin D<sub>3</sub>, which is then transformed to vitamin D<sub>3</sub> by an isomerization process. Vitamin D<sub>3</sub> subsequently undergoes hydroxylation

Table 1. Response of Various Target Cells to Vitamin D

Potential targets of vitamin D	Effector function of vitamin D
Lung bronchial smooth muscle cells	Inhibit cytokine synthesis and release: Decrease lung inflammation (eg, RANTES) Inhibit bronchial smooth muscle cell proliferation and remodeling (eg, MMP-9, MMP-33, PDGF)
Mast cells	Inhibit the differentiation, maturation, and homing of mast cells to allergic airways
Dendritic cells	Downregulate expression of costimulatory molecules CD40 and CD80/CD86 Enhance IL-10 synthesis
Regulatory T cells	Enhance IL-10 synthesis Enhance TGF- $\beta$ synthesis

Abbreviations: IL, interleukin; MMP, metalloproteinase; PDGF, platelet-derived growth factor; TGF- $\beta$ , transforming growth factor  $\beta$ .

tion in the liver by 25-hydroxylase to its major circulating metabolite 25(OH)D3 and then in the kidney by  $1\alpha$ -hydroxylase to its biologically active form 1,25-dihydroxyvitamin D3 (1,25[OH]2D3) (Figure 1).<sup>6</sup> However, recent studies indicate that  $1\alpha$ -hydroxylase is present in many other cells, including lung cells. The exact values that constitute vitamin D deficiency are under debate. It is generally accepted that 25(OH)D3 levels less than 10 to 12 ng/mL represent deficiency, levels less than 20 ng/mL represent insufficiency, and levels greater than 30 ng/mL indicate sufficiency.

Vitamin D is a unique molecule; historically, humans naturally obtain 90% of vitamin D from sunlight exposure and 10% from diet. This has changed because many foods are now fortified with vitamin D, such as milk and milk products, margarine, and breakfast cereals. Vitamin D is not endogenous to most foods, except perhaps fish liver oils (eg, cod liver oil), eggs, and beef liver.<sup>6</sup> Despite fortification of foods, many studies have shown a relative vitamin D insufficiency in westernized society. Binkely et al<sup>7</sup> conducted a study in a population from Hawaii consisting of 93 adults (30 women and 63 men) with a mean age and body mass index (calculated as weight in kilograms divided by height in meters squared) of 24 years and 23.6, respectively. Their self-reported sun exposure was 28.9 hours per week, yielding a calculated sun exposure index of 11.1. Serum 25(OH)D3 concentration was measured, and a low vitamin D status was defined as less than 30 ng/mL. The mean serum 25(OH)D3 concentration was

31.6 ng/mL. Fifty-one percent of this population had low vitamin D status despite abundant sun exposure.

The true level of vitamin D deficiency in American children and adolescents is unknown, but estimates of 30% to 80% have been reported.<sup>8</sup> In a recent study researchers analyzed data on more than 6,000 children, ages 1 to 21 years, collected by NHANES, 2001-2004. They found that 9% of the study sample, equivalent to 7.6 million children across the United States, was vitamin D deficient (defined as an 25[OH]D3 level <15 ng/mL of blood), whereas another 61%, or 50.8 million, was vitamin D insufficient (15-29 ng/mL). Low vitamin D levels were especially common in children who were older, female, African American, and Mexican American; drank milk less than once a week; or spent more than 4 hours a day watching television, playing videogames, or using computers.<sup>9</sup> Obesity was also noted to be a risk factor for lower serum 25(OH)D3 levels. On the basis of these and other data, it appears that vitamin D deficiency or insufficiency rates in the United States are high.

## GENETICS

The notion that vitamin D receptor (VDR) polymorphisms could be associated with disease has been suggested previously in immune and inflammatory conditions, such as Crohn disease and tuberculosis. Genome scans for asthma have identified possible linkages on 17 different chromosomes, including chromosome 12, region q13-23. Because the VDR is mapped to chromosome 12q, some geneticists have postulated an association between VDR polymorphisms and ge-

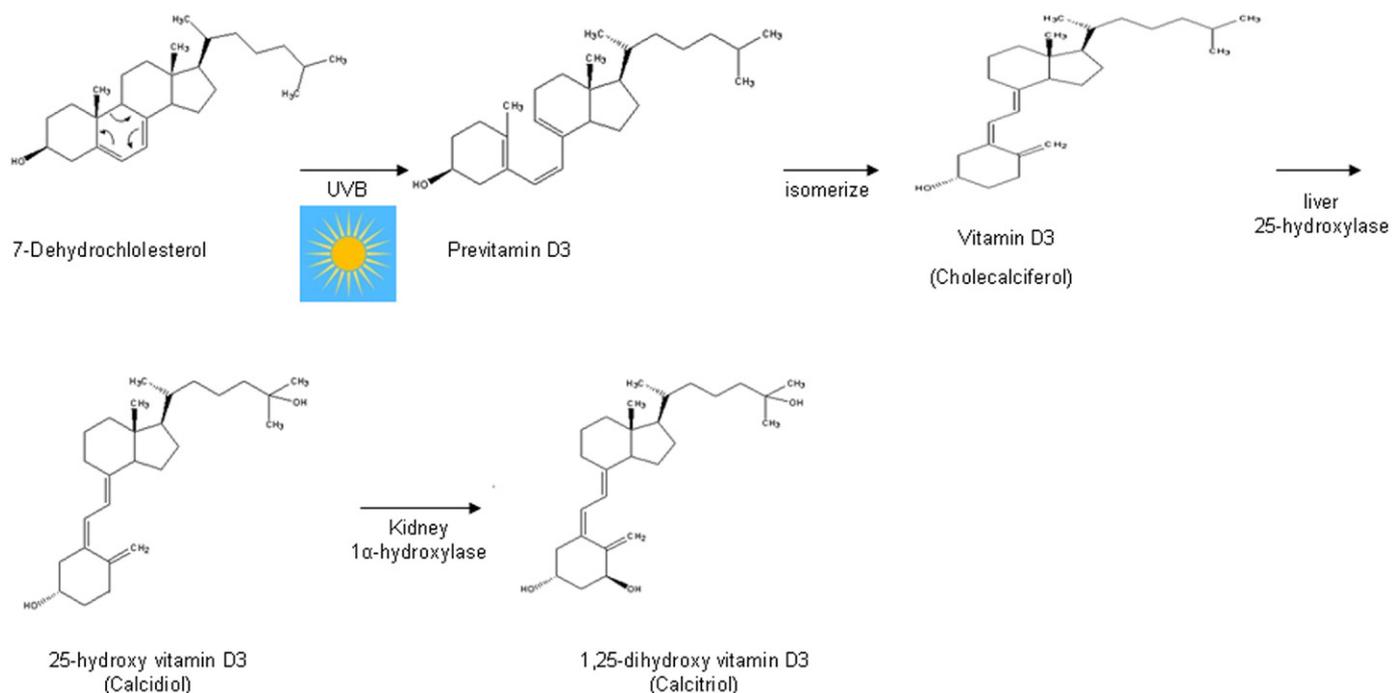


Figure 1. Biosynthesis pathway of vitamin D.

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netic susceptibility for asthma.<sup>10–13</sup> A study published in 2004 examined VDR genetic variants in an asthma family-based cohort from Quebec, Canada.<sup>13</sup> They identified 6 variants to be strongly associated with asthma and 4 with atopy. A separate genetic study by Raby et al<sup>10</sup> also found 4 of 6 VDR variants with significant association to asthma. However, other authors have not found this association.<sup>12</sup> Thus, the possibility of VDR polymorphisms playing a role in the relationship between asthma and vitamin D remains to be clarified.

#### VITAMIN D AND AIRWAY EPITHELIUM

Studies have shown that a number of tissues express 1 $\alpha$ -hydroxylase and are able to activate vitamin D. Hansdottir et al<sup>14</sup> demonstrated that lung epithelial cells express high baseline levels of 1 $\alpha$ -hydroxylase. This allows airway epithelial cells to convert inactive 25(OH)D3 to the active 1,25(OH)2D3 locally within the lung.

#### VITAMIN D AND BRONCHIAL SMOOTH MUSCLE

Considering the prominent role of bronchial smooth muscle cells in airway remodeling, luminal narrowing, and bronchoconstriction, Bosse et al<sup>15</sup> examined whether the VDR is present. They found that VDR was present in bronchial smooth muscle at both the messenger RNA transcript and protein levels. The functionality of the receptor was then demonstrated by showing a 200-fold change in the expression of the 24-hydroxylase gene after calcitriol (1,25[OH]2D3) stimulation. 24-Hydroxylase regulates vitamin D homeostasis by initiating the degradation of 1,25(OH)D3. Similarly, Damera and colleagues<sup>16</sup> showed that calcitriol increased expression of 24-hydroxylase on bronchial smooth muscle. They also demonstrated that calcitriol, but not dexamethasone, markedly inhibited platelet-derived growth factor-induced bronchial smooth muscle DNA synthesis in a dose-dependent manner, with a maximum inhibitory effect of 60% (n = 12) at 100nM calcitriol.

A recent study by Banerjee et al<sup>17</sup> also suggested a role for vitamin D in bronchial smooth muscle. Bronchial smooth muscle cells were treated with tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) and/or interferon  $\gamma$  (IFN- $\gamma$ ) for 24 hours in the presence of calcitriol and/or the glucocorticoid fluticasone. In TNF- $\alpha$ -treated cells, calcitriol inhibited RANTES (CCL5) and interferon inducible protein 10 secretion in a concentration-dependent manner. In TNF- $\alpha$ /IFN- $\gamma$ -treated cells, the combination of both TNF- $\alpha$  and IFN- $\gamma$  additively inhibited RANTES secretion (by 60%), whereas fluticasone or calcitriol alone inhibited RANTES secretion by only 38% and 20%, respectively.

Song et al<sup>18</sup> investigated the effect of calcitriol on passively sensitized human bronchial smooth muscle cell proliferation and matrix metalloproteinases (MMPs) 9 and 33 (ADAM33) expression. MMPs are believed to play a role in airway remodeling. They found that 1,25(OH)2D3 effectively suppressed passively sensitized bronchial smooth muscle cell proliferation, G1/S transition, in addition to downregulating

the expression of MMP-9 and MMP-33 (ADAM33). These data from Bosse et al, Damera et al, Banerjee et al, and Song et al suggest that lower vitamin D levels could lead to bronchial smooth muscle proliferation, cytokine release, and thus airway remodeling. However, Bosse and his group<sup>15</sup> also used microarray technology and real-time polymerase chain reaction to look at gene expression after stimulation with 1,25(OH)2D3. Their results were mixed. For example, they demonstrated that 1,25(OH)2D3 increased the expression of prostaglandin (PG) F synthase (AKR1C3) in bronchial smooth muscle, which is believed to play an important role in the pathogenesis of allergic diseases by regulating the synthesis of PGs, such as PGD<sub>2</sub> and PGF<sub>2</sub>. PGF synthase in human lung catalyzes the reduction of PGD<sub>2</sub> to PGF<sub>2</sub> at low pH and the oxidation PGF<sub>2</sub> to PGD<sub>2</sub> at high pH.<sup>19</sup> PGD<sub>2</sub> is a lipid mediator that directs the earliest phases of T-cell recruitment to the airways immediately after exposure to allergens.<sup>20</sup> Furthermore, PGF synthase is known to inactivate cortisol.<sup>21</sup> They also found increased transcription of a Rho guanosine triphosphatase, which can allow the guanosine triphosphate binding protein Rho A to activate its downstream target Rho-kinase, leading to a procontractile state. These data suggest that vitamin D deficiency may be associated with beneficial rather than detrimental effects for asthma. The involvement of Rho A in vitamin D-mediated signal transduction is further supported by data from Khare et al,<sup>22</sup> who investigated the mechanism of phospholipase D activation by vitamin D in Caco-2 cells. Treatment with C3 transferase, which inhibits members of the Rho family of monomeric G proteins, markedly diminished the ability of 1,25(OH)2D3 to stimulate phospholipase D. In addition, Ordóñez-Morán and colleagues<sup>23</sup> established that 1,25(OH)2D3 induces calcium influx and activation of Rho A, which was VDR dependent.

#### VITAMIN D AND VASCULAR SMOOTH MUSCLE

Another important aspect of airway remodeling is angiogenesis. Mitsuhashi et al<sup>24</sup> evaluated whether 1,25(OH)2D3 affects the growth rate of neonatal rat vascular smooth muscle cells. They found that the effect of 1,25(OH)2D3 depends crucially on the context in which it is added. In nonquiescent cells, 1,25(OH)2D3 blunted the growth response to thrombin and platelet-derived growth factor, as well as the induction of c-myc RNA by thrombin. Conversely, in quiescent cells 1,25(OH)2D3 enhanced both the growth response and the induction of c-myc RNA by thrombin.

#### IMMUNOMODULATORY EFFECT OF VITAMIN D

In addition to its role on airway epithelium and bronchial smooth muscle, vitamin D may also be involved in asthma pathogenesis through its effects on the immune system. The discovery of the high-affinity receptor for 1,25(OH)2D3 on monocytes and lymphocytes has also added fuel to this hypothesis.<sup>25</sup> Vitamin D has been shown to have significant immunomodulatory effects by interacting with multiple immune cells involved in asthma, including mast cells,<sup>26</sup> CD4<sup>+</sup> T cells of both the T<sub>H</sub>1 and T<sub>H</sub>2 phenotype,<sup>27–31</sup> monocytes,

macrophages, dendritic cells (DCs),<sup>32–35</sup> and regulatory T cells (Tregs).<sup>36–41</sup>

Mast cells play an important role in inflammatory and allergic responses and are one of the main effector cells of the T<sub>H</sub>2 response. Cross-linking of the high-affinity IgE receptor FcεRI by antigens bound to IgE antibodies provides the main immunologic stimulus for mast cell activation. Mast cells have been shown to be increased in the airways of asthmatic patients compared with healthy controls.<sup>42</sup> Baroni et al<sup>26</sup> demonstrated that 1,25(OH)2D3 contributes to the regulation of development and function of mast cells. They found that 1,25(OH)2D3 promotes apoptosis and inhibits maturation of mouse bone marrow–derived mast cell precursors. There was also a dose-dependent inhibition of mast cell differentiation by 1,25(OH)2D3 at various stages of mast cell development. This appeared to be VDR dependent because mast cell progenitors obtained from VDR-ablated mice underwent an accelerated maturation *in vitro* and gave rise to more responsive mast cells than wild-type mice. They also demonstrated histologically a moderate increase in the number of mast cells in the skin of VDR-deficient mice compared with wild-type mice. On the basis of these data, it is tempting to postulate that vitamin D may play an important inhibitory role in the differentiation, maturation, and homing of mast cells to allergic airways.

Many authors have shown an interaction between vitamin D and CD4<sup>+</sup> T cells. Reichel et al<sup>28</sup> demonstrated that 1,25(OH)2D3 inhibited synthesis of the T<sub>H</sub>1 cytokine IFN-γ by phytohemagglutinin-activated human peripheral blood lymphocytes. 1,25(OH)2D3 also inhibited accumulation of IFN-γ messenger RNA in activated peripheral blood lymphocytes in a dose-dependent fashion.

The timing of vitamin D exposure (or lack thereof) may have large implications on how immune cells respond to vitamin D. Cord blood cells contain mainly naïve T cells expressing the CD45RA phenotype, which can behave differently from adult peripheral blood T cells. Lee et al<sup>45</sup> found a high correlation between maternal vitamin D levels and cord blood levels in the fetus. Fifty percent of the mothers and 65% of their newborn infants from an inner-city hospital were vitamin D deficient or insufficient, with levels less than 30 ng/mL. Camargo et al<sup>46</sup> analyzed a prospective prebirth cohort study in Massachusetts consisting of 1,194 mother-child pairs. They assessed the maternal intake of vitamin D during pregnancy from a validated food frequency questionnaire. The primary outcome was recurrent wheeze by the age of 3 years. The mean total vitamin D intake during pregnancy was 548 IU/d. By 3 years of age, 186 children (16%) had recurrent wheeze. Compared with mothers in the lowest quartile of daily intake (median, 356 IU), those in the highest quartile (724 IU) had a lower risk of having a child with recurrent wheeze (odds ratio, 0.39; 95% confidence interval, 0.25–0.62). These data are consistent with those of Pichler et al,<sup>29</sup> who evaluated the effects of 1,25(OH)2D3 on human naïve cord blood T lymphocytes and their differentiation into T<sub>H</sub>1- or T<sub>H</sub>2-type cells. Their results showed that

1,25(OH)2D3 not only inhibits IL (interleukin) 12–generated IFN-γ production but also suppressed IL-4–induced IL-4 and IL-13 expression. Thus, it appears that 1,25(OH)2D3 inhibits not only T<sub>H</sub>1 differentiation, as in T cells from adults, but also suppresses T<sub>H</sub>2 differentiation in cord blood T cells.

Topilski et al<sup>30</sup> investigated the role of vitamin D in the experimental T<sub>H</sub>2 model of ovalbumin sensitized asthmatic mice. Ovalbumin inhalation induced infiltration of eosinophils (64% vs 0.1% control; *P* < .001) and lymphocytes (7.7% vs 1.1% control; *P* = .005) into the bronchoalveolar lavage fluid (BALF) of sensitized mice. However, pretreatment with 1,25(OH)2D3 intraperitoneally significantly inhibited antigen-induced eosinophil recruitment into the BALF (64% ovalbumin vs 9.8% vitamin D<sub>3</sub> and ovalbumin, *P* < .001). In addition, IL-4 levels in BALF derived from 1,25(OH)2D3-treated mice were reduced compared with the ovalbumin alone (33 pg/mL of ovalbumin vs 8 pg/mL of vitamin D and ovalbumin, *P* = .007). This trend toward inhibition was maintained even when 1,25(OH)2D3 was injected 2 weeks after mice were sensitized with ovalbumin. These findings suggest that 1,25(OH)2D3 significantly reduces the airway inflammatory response and IL-4 levels in BALF when given at the initiation of sensitization or after the CD4<sup>+</sup> population was already skewed towards the T<sub>H</sub>2 population. Topilski et al also found that 1,25(OH)2D3 inhibits chemotaxis and actin polymerization by CD4<sup>+</sup> T cells, suggesting that in addition to its role in cytokine production it also may be involved in homing of T cells to lymph nodes or tissues.

Matheu et al<sup>27</sup> used a similar murine model of pulmonary eosinophilia with ovalbumin sensitized mice to evaluate the effects of vitamin D on a T<sub>H</sub>2 profile. Five-week-old mice were primed on day 0 with ovalbumin intraperitoneally. Then they were nasally challenged with ovalbumin on days 7, 8, 9, 10, and 11. Some mice received subcutaneous injections of vitamin D. Their experiments yielded mixed results. Although treatment with vitamin D augmented allergen-induced T<sub>H</sub>2 cytokines (IL-4 and IL-13) and IgE production, the local inflammatory response in BALF was significantly decreased with impaired recruitment of eosinophils and lower levels of IL-5. Matheu et al attributed these findings to late treatment with vitamin D after establishment of an early immune response. However, as noted herein, Topilski et al showed that inhibition of eosinophilic recruitment was maintained even when 1,25(OH)2D3 was injected 2 weeks after mice were ovalbumin sensitized. Despite these differing results, both Topilski et al and Matheu et al found that vitamin D decreased the levels of eosinophils and T<sub>H</sub>2 cytokines in BALF of ovalbumin sensitized mice. However, seemingly contradictory evidence to these findings comes from VDR knockout mice that failed to develop airway inflammation, eosinophilia, or airway hyperresponsiveness after ovalbumin sensitization.<sup>43,44</sup>

Boonstra et al<sup>31</sup> evaluated the effects of vitamin D on an *in vitro* model from mice transgenic for an ovalbumin-specific α/β T-cell receptor. They cultured these cells with splenic

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antigen-presenting cells and ovalbumin peptide with medium alone or medium supplemented with 1,25(OH)2D3. Cytokine production was determined by flow cytometry on restimulation. As in other models described herein, the T<sub>H</sub> cells showed a reduction in the number of IFN- $\gamma$ -producing cells. However, similar to Matheu et al, they also noted an increased frequency of IL-4-producing cells. The vitamin D-induced effects were thought to be largely mediated via IL-4 because neutralization of IL-4 with a monoclonal antibody almost completely abrogated T<sub>H</sub>2 cell development. In addition, the increased production of T<sub>H</sub>2 cytokines correlated with increased expression of the T<sub>H</sub>2-specific transcription factor GATA-3.

When we examine all the data on vitamin D using the experimental murine model for asthma, it appears as though vitamin D decreases the peripheral blood T<sub>H</sub>1 profile and increases the T<sub>H</sub>2 profile. Seemingly contradictory to this T<sub>H</sub>1-T<sub>H</sub>2 skewing is the reduced local inflammatory response seen in the murine lung in vitamin D-treated mice. In particular, the BALF has consistently been shown to have decreased eosinophils and T<sub>H</sub>2 cytokines. The question that is raised is why do ovalbumin sensitized mice treated with vitamin D have decreased airway inflammation when VDR knockout mice also do not develop airway inflammation on ovalbumin exposure. One possibility is that knocking out the VDR in all tissues can alter the homeostasis of calcium and have widespread effects on not only immune cells but also all cells of the body, especially because the VDR is expressed in many tissues and cells. This also underlies the inherent limitations of using murine models for explaining human disease and does not take into consideration the timing of exposure of vitamin D or the effects of Tregs to this model.

Antigen-presenting cells, such as DCs, express the VDR and are key targets of VDR agonists. Hewison et al<sup>32</sup> and his group demonstrated that monocyte-derived DCs are able to synthesize 1,25(OH)2D3 in vitro as a consequence of increased 1 $\alpha$ -hydroxylase expression. Numerous studies have demonstrated that 1,25(OH)2D3 and its analogs inhibit the differentiation and maturation of DCs.<sup>47–51</sup> These studies have shown that in vitro treatment of DCs with 1,25(OH)2D3 and its analogs leads to downregulated expression of the costimulatory molecules CD40 and CD80/CD86 and to decreased IL-12 and enhanced IL-10 production, resulting in decreased T-cell activation. The block of maturation, coupled with abrogation of IL-12 and strongly enhanced production of IL-10, may explain the capacity of VDR agonists to induce DCs with tolerogenic properties that favor regulatory T-cell enhancement.

Vitamin D has been shown to promote the induction of Treg cells.<sup>37,38,40,41</sup> IL-10-secreting Tregs are of particular importance. IL-10 is a potent antiinflammatory cytokine and inhibits T<sub>H</sub>1 and T<sub>H</sub>2 immune responses, which has led to considerable interest in its role in allergic inflammation. Convincing experiments for this interaction comes from Xystrakis et al.<sup>36</sup> and Urry et al.<sup>39</sup> They showed that human CD4<sup>+</sup> Tregs secrete higher levels of IL-10 when stimulated in the

presence of dexamethasone and vitamin D3.<sup>36</sup> Furthermore, after stimulation by allergen, IL-10-secreting Tregs inhibited cytokine secretion by allergen-specific T<sub>H</sub>2 cells in an IL-10-dependent manner. Xystrakis et al also looked at glucocorticoid-resistant asthma. They cultured CD4<sup>+</sup> T cells from patients with glucocorticoid-resistant asthma and found that dexamethasone did not enhance secretion of IL-10. The addition of vitamin D3 with dexamethasone to cultures of these CD4<sup>+</sup> T cells enhanced IL-10 synthesis to levels observed in cells from glucocorticoid-sensitive patients cultured with dexamethasone alone.<sup>39</sup> The role of vitamin D in IL-10 synthesis is supported by findings that vitamin D can also increase the expression of IL-10 by DCs. Taher et al<sup>52</sup> investigated whether the combination of allergen immunotherapy with 1,25(OH)2D3 in mice can potentiate the suppressive effects of immunotherapy and whether IL-10 and TGF- $\beta$  are involved. Interestingly, when compared with immunotherapy alone, coadministration of 10 ng of 1,25(OH)2D3 with 100 $\mu$ g of ovalbumin immunotherapy significantly inhibited airway hyperresponsiveness and decreased serum ovalbumin-specific IgE levels, airway eosinophilia, and T<sub>H</sub>2-related cytokines. This correlated with increased IL-10 and TGF- $\beta$  levels in lung tissues. The suppressive effect of this combined immunotherapy was completely inhibited by treatment with monoclonal antibodies to IL-10 receptor and TGF- $\beta$ .

## VITAMIN D AND RESPIRATORY INFECTIONS

Respiratory viral infections are important initiators of asthma exacerbations. Recent data suggest that vitamin D reduces the risk of respiratory viral infections. In a randomized controlled trial conducted with postmenopausal African American women living in Long Island, New York, those taking 800 IU/d of vitamin D had a 60% reduction in the incidence of seasonal influenza or common cold. However, those taking 2,000 IU/d had a 90% reduction.<sup>53</sup> On the basis of these and other studies, it was suggested that differences in skin pigmentation and, thus, vitamin D production explained the variations in risk of childhood respiratory infections in Hawaii.<sup>54</sup> Additional support for the role of vitamin D is provided by data showing that it is capable of inducing the expression of several anti-infective molecules, such as cathelicidin. Thus, deficiency of vitamin D might lead to greater occurrences of respiratory tract infections, and consequently, more asthma exacerbations.

## CONCLUSIONS

One critical issue in assessing the role of vitamin D in asthma is that there is no uniform agreement on what constitutes vitamin D sufficiency, insufficiency, and deficiency. It is generally accepted that serum 25(OH)D3 levels less than 10 to 12 ng/mL represent deficiency, levels less than 20 ng/mL represent insufficiency, and levels greater than 30 ng/mL represent sufficiency. However, a review of existing studies shows associations between airway diseases and serum 25(OH)D3 using only a single

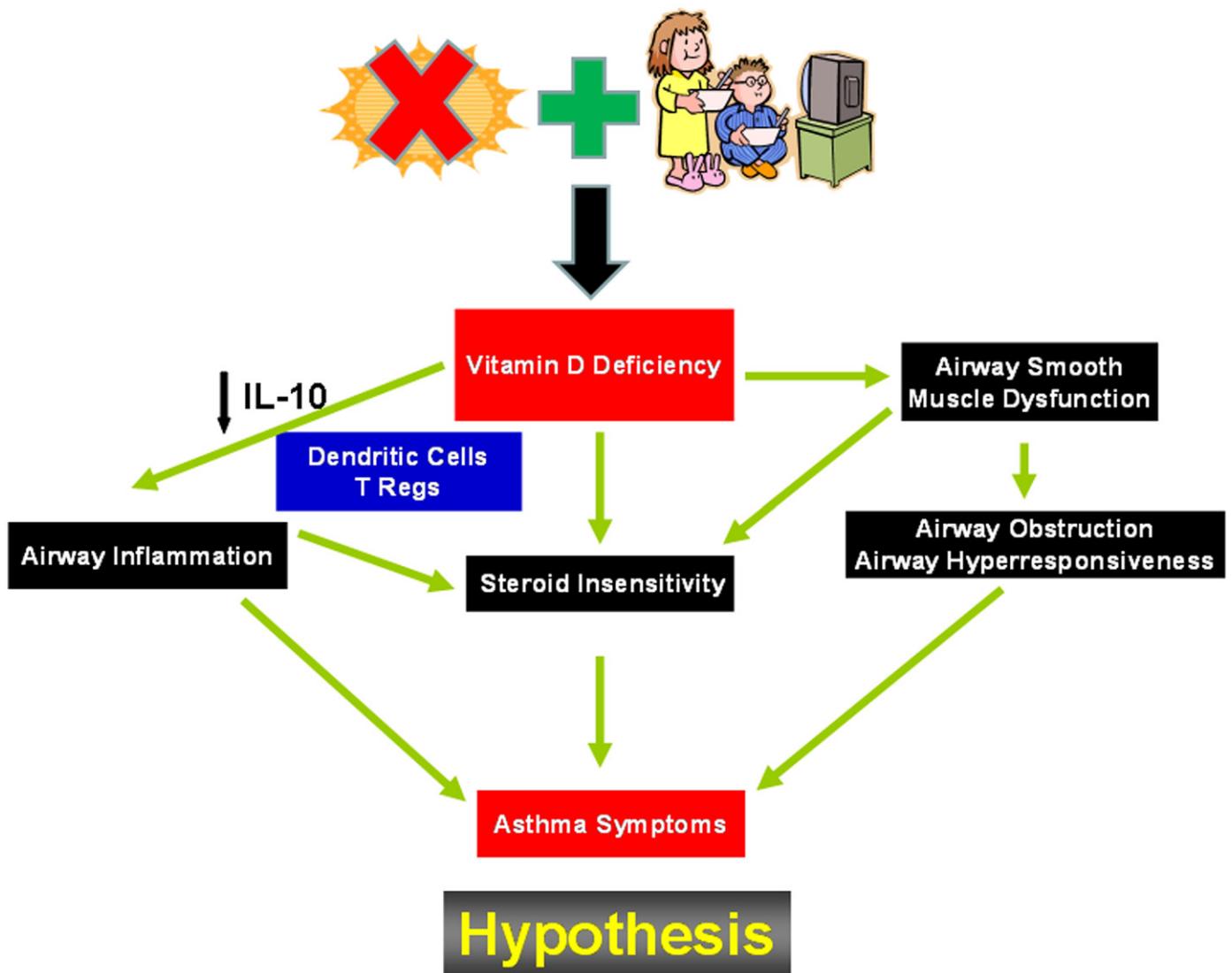


Figure 2. Hypothesis demonstrating the various effects of vitamin D deficiency on asthma pathogenesis. IL indicates interleukin; T regs, regulatory T cells.

measurement. This can result in unreliable data because serum 25(OH)D3 levels usually increase by 5 to 10 ng/mL between winter and summer (Christopher Gallagher, MD, unpublished data, September 2009). For example, an individual may be vitamin D deficient in April but vitamin D sufficient in June because of sun exposure. Thus, in conducting studies examining the relationship between serum 25(OH)D3 levels and asthma, more frequent measurements of serum 25(OH)D3 should be performed to adjust for the seasonal variation. More importantly, there are no data defining what constitutes appropriate levels of vitamin D in target organs, such as lung. Dose-response data on the effects of vitamin D in raising levels in the serum and treating diseases such as asthma are clearly needed to best define the role of vitamin D in asthma.

Studies such as the NHANES trial suggest an association between vitamin D deficiency and asthma. However, these studies suffer from lack of long-term follow-up of patients, and only a single measurement of serum 25(OH)D3 was obtained, which was uncorrected for season. Further studies are needed to investigate the relationship between vitamin D levels and parameters for atopy and asthma using frequent blood sampling.

We hypothesize that vitamin D supplementation will lead to better asthma control by inhibiting the influx of inflammatory cytokines in the lung and increasing the secretion of IL-10 by CD4<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> Treg cells and DCs, while concurrently inhibiting DC activation and maturation. This block of maturation, coupled with enhanced production of IL-10, may explain the capacity of VDR agonists to induce

DCs with tolerogenic properties that favor Treg enhancement (Figure 2). Furthermore, early vitamin D supplementation to the mother and neonate may be paramount in terms of its effect on the development of a T<sub>H</sub>1 or T<sub>H</sub>2 phenotype in the child. Proof of this hypothesis requires carefully designed long-term interventional trials with vitamin D in patients with asthma.

## REFERENCES

- Masoli M, Fabian D, Holt S, Beasley R; Global Initiative for Asthma (GINA) Program. The global burden of asthma: executive summary of the GINA Dissemination Committee report. *Allergy*. 2004;59:469–478.
- Brehm JM, Celedo JC, Soto-Quiros ME, et al. Serum vitamin D levels and markers of severity of childhood asthma in Costa Rica. *Am J Respir Crit Care Med*. 2009;179:765–771.
- Bateman ED, Boushey HA, Bousquet J, et al. Can guideline-defined asthma control be achieved? the Gaining Optimal Asthma Control Study. *Am J Respir Crit Care Med*. 2004;170:836–844.
- Utz G, Hauck AM. Oral application of calcium and vitamin D2 in allergic bronchial asthma. *MMW Munch Med Wochenschr*. 1976;118:1395–1398.
- Black PN, Scragg R. Relationship between serum 25-hydroxyvitamin D and pulmonary function in the third national health and nutrition survey. *Chest*. 2005;128:3792–3798.
- Lamberg-Allardt C. Vitamin D in foods and as supplements. *Prog Biophys Mol Biol*. 2006;92:33–38.
- Binkley N, Novotny R, Krueger D, et al. Low vitamin D status despite abundant sun exposure. *J Clin Endocrinol Metab*. 2007;92:2130–2135.
- Saintonge S, Bang H, Gerber L. Implications of a New definition of vitamin D deficiency in a multiracial US adolescent population: the National Health and Nutrition Examination Survey III. *Pediatrics*. 2009;123:797–803.
- Kumar J, Muntner P, Kaskel FJ, Hailpern SM, Melamed ML. Prevalence and associations of 25-hydroxyvitamin D deficiency in US children: NHANES 2001–2004. *Pediatrics*. In press.
- Raby BA, Lazarus R, Silverman EK, et al. Association of vitamin D receptor gene polymorphisms with childhood and adult asthma. *Am J Respir Crit Care Med*. 2004;170:1057–1065.
- Wjst M, Altmuller J, Faus-Kessler T, Braig C, Bahnweg M, Andre E. Asthma families show transmission disequilibrium of gene variants in the vitamin D metabolism and signaling pathway. *Respir Res* 2006;7:60.
- Vollmert C, Illig T, Altmuller J, et al. Single nucleotide polymorphism screening and association analysis: exclusion of integrin b7 and vitamin D receptor (chromosome 12q) as candidate genes for asthma. *Clin Exp Allergy*. 2004;34:1841–1850.
- Poon AH, Laprise C, Meire M, et al. Association of vitamin D receptor genetic variants with susceptibility to asthma and atopy. *Am J Respir Crit Care Med*. 2004;170:967–973.
- Hansdottir S, Monick MM, Hinde SL, Lovan N, Look DC, Hunninghake GW. Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defense 1. *J Immunol*. 2008;181:7090–7099.
- Bosse Y, Maghni K, Hudson TJ. 1 $\alpha$ , 25-dihydroxyvitamin D<sub>3</sub> stimulation of bronchial smooth muscle cells induces autocrine, contractility and remodeling processes. *Physiol Genomics*. 2007;29:161–168.
- Damera G, Fogle H, Goncharova EA, Zhao H, Krymskaya VP, Panetier RA. Vitamin D attenuates growth factor-induced human airway smooth muscle cell proliferation. In: Proceedings from the ATS 2009 International Conference; May 15-20, 2009; San Diego, California. Abstract 2723.
- Banerjee A, Damera G, Bhandere R, Gu S, Lopez-Boado YS, Tliba O. Vitamin D and glucocorticoids differentially modulate chemokine expression in human airway smooth muscle cells. *Br J Pharmacol*. 2008;155:84–92.
- Song Y, Qi H, Wu C. Effect of 1, 25-(OH) 2D3 (a vitamin D analogue) on passively sensitized human airway smooth muscle cells. *Respirology*. 2007;12:486–494.
- Suzuki-Yamamoto T, Nishizawa M, Fukui M, et al. cDNA cloning, expression and characterization of human prostaglandin F synthase. *FEBS Lett*. 1999;462:335–340.
- Luster AD, Tager AM. T-cell trafficking in asthma: lipid mediators grease the way. *Nat Rev Immunol*. 2004;4:711–724.
- Sharma KK, Lindqvist A, Zhou XJ, Auchus RJ, Penning TM, Andersson S. Deoxycorticosterone inactivation by AKR1C3 in human mineralocorticoid target tissues. *Mol Cell Endocrinol*. 2006;248:79–86.
- Khare S, Bissonnette M, Wali R, et al. 1-,25-Dihydroxyvitamin D3 but not TPA activates PLD in Caco-2 cells via pp60c-src and RhoA. *Am J Physiol Gastrointest Liver Physiol*. 1999;276:G993–G1004.
- Ordóñez-Morán P, Larriba MJ, Pálmer HG, et al. RhoA – ROCK and p38MAPK-MSK1 mediate vitamin D effects on gene expression, phenotype, and Wnt pathway in colon cancer cells. *J Cell Biol*. 2008;183:697–710.
- Mitsuhashi T, Morris RC, Ives HE. 1, 25-dihydroxyvitamin D3 modulates growth of vascular smooth muscle cells. *J Clin Invest*. 1991;87:1889–1895.
- Provvedini DM, Tsoukas CD, Deftos LJ, Manolagas SC. 1,25-dihydroxyvitamin D3 receptors in human leukocytes. *Science*. 1983;221:1181–1183.
- Baroni E, Biffi M, Benigni F, et al. VDR-dependent regulation of mast cell maturation mediated by 1,25-dihydroxyvitamin D<sub>3</sub>. *J Leukoc Biol*. 2007;81:250–262.
- Matheu V, Bäck O, Mondoc E, Issazadeh-Navikas S. Dual effects of vitamin D-induced alteration of T<sub>H</sub>1/T<sub>H</sub>2 cytokine expression: enhancing IgE production and decreasing airway eosinophilia in murine allergic airway disease. *J Allergy Clin Immunol*. 2003;112:585–92.
- Reichel H, Koeffler HP, Tobler A, Norman AW. 1 alpha, 25-Dihydroxyvitamin D3 inhibits gamma-interferon synthesis by normal human peripheral blood lymphocytes. *Proc Natl Acad Sci U S A*. 1987;84:3385–3389.
- Pichler J, Gerstmayr M, Szepefalusi Z, et al. 1 alpha,25(OH)2D3 inhibits not only Th1 but also Th2 differentiation in human cord blood T cells. *Pediatr Res*. 2002;52:12–18.
- Topilski I, Flaishon L, Naveh Y, et al. The anti-inflammatory effects of 1,25-dihydroxyvitamin D3 on Th2 cells in vivo are due in part to the control of integrin-mediated T lymphocyte homing. *Eur J Immunol*. 2004;34:1068–1076.
- Boonstra A, Barrat FJ, Crain C, et al. 1alpha,25-Dihydroxyvitamin d3 has a direct effect on naive CD4(β) T cells to enhance the development of Th2 cells. *J Immunol*. 2001;167:4974–4980.
- Hewison M, Freeman L, Hughes SV, et al. Differential regulation of vitamin D receptor and its ligand in human monocyte-derived dendritic cells. *J Immunol*. 2003;170:5382–5390.
- Fargeas C, Wu C, Luo H, Sarfati M, Delespesse G, Wu J. 1,25 (OH)<sub>2</sub> Vitamin D<sub>3</sub> inhibits the CD23 gene expression by human peripheral blood monocytes. *J Immunol*. 1990;145:4053–4058.
- Adorini L, Penna G, Giarratana N, et al. Dendritic cells as key targets for immunomodulation by vitamin D receptor ligands. *J Steroid Biochem Mol Biol*. 2004;89–90:437–441.
- Iho S, Kura F, Sugiyama H, et al. The role of monocytes in the suppression of PHA-induced proliferation and IL 2 production of human mononuclear cells by 1,25-dihydroxyvitamin D3. *Immunol Lett*. 1985;11:331–336.
- Xystrakis E, Kusumakar S, Boswell S, et al. Reversing the defective induction of IL-10-secreting regulatory T cells in glucocorticoid-resistant asthma patients. *J Clin Invest*. 2006;116:146–155.
- Penna G, Roncari A, Amuchastegui S, et al. Expression of the inhibitory receptor ILT3 on dendritic cells is dispensable for induction of CD4+Foxp3+ regulatory T cells by 1, 25-dihydroxyvitamin D3. *Blood*. 2005;106:3490–3497.
- Ghoreishi M, Bach P, Obst J, Kmoba M, Fleet JC, Dutz JP. Expansion of antigen-specific regulatory T cells with the topical vitamin D analog calcipotriol. *J Immunol*. 2009;182:6071–6078.

- 
39. Urry Z, Xystrakis E, Richards DF, et al. Ligation of TLR9 induced on human IL-10 secreting Tregs by  $1\alpha,25$ -dihydroxyvitamin D3 abrogates regulatory function. *J Clin Invest*. 2009;119:387–398.
40. Gorman S, Kuritzky LA, Judge MA, et al. Topically applied  $1,25$ -dihydroxyvitamin D3 enhances the suppressive activity of CD4+CD25+ cells in the draining lymph nodes. *J Immunol*. 2007;179:6273–6283.
41. McGlade JP, Gorman S, Zosky GR, et al. Suppression of the asthmatic phenotype by ultraviolet B-induced, antigen-specific regulatory cells. *Clin Exp Allergy*. 2007;37:1267–1276.
42. Rightling CEB, Bradding P. Mast-cell infiltration of airway smooth muscle in asthma. *N Engl J Med*. 2002;346:1699–1705.
43. Wittke A, Chang A, Froicu M, et al. Vitamin D receptor expression by the lung micro-environment is required for maximal induction of lung inflammation. *Arch Biochem Biophys*. 2007;460:306–313.
44. Wittke A, Weaver V, Mahon BD, August A, Cantorna MT. Vitamin D receptor-deficient mice fail to develop experimental allergic asthma. *J Immunol*. 2004;173:3432–3436.
45. Lee JM, Smith JR, Philipp BL, et al. Vitamin D deficiency in a healthy group of mothers and newborn infants. *Clin Pediatr (Phila)*. 2007;46:42–44.
46. Camargo CA, Rifas-Shiman SL, Litonjua AA, et al. Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in children a 3 y of age. *Am J Clin Nutr*. 2007;85:788–795.
47. Penna G, Adorini L.  $1\alpha,25$ -dihydroxyvitaminD3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. *J Immunol*. 2000;164:2405–2411.
48. Piemonti L, Monti P, Sironi M, et al. Vitamin D3 affects differentiation, maturation, and function of human monocyte-derived dendritic cells. *J Immunol*. 2000;164:4443–4451.
49. Griffin MD, Lutz WH, Phan VA, Bachman LA, McKean DJ, Kumar R. Potent inhibition of dendritic cell differentiation and maturation by vitamin D analogs. *Biochem Biophys Res Commun*. 2000;270:701–708.
50. van Halteren AG, van Etten E, de Jong EC, Bouillon R, Roep BO, Mathieu C. Redirection of human autoreactive T-cells upon interaction with dendritic cells modulated by TX527, an analog of  $1,25$  dihydroxyvitamin D(3). *Diabetes*. 2002;51:2119–2125.
51. Gauzzi MC, Purificato C, Donato K, et al. Suppressing effect of  $1\alpha,25$ -dihydroxyvitamin D3 on type I IFN-mediated monocyte differentiation into dendritic cells: impairment of functional activities and chemotaxis. *J Immunol*. 2005;174:270–276.
52. Taher YA, van Esch BCAM, Hofman GA, Henricks PAJ, van Oosterhout AJM.  $1\alpha, 25$ -dihydroxyvitamin D<sub>3</sub> potentiates the beneficial effects of allergen immunotherapy in a mouse model of allergic asthma: role for IL-10 and TGF- $\beta$ . *J Immunol*. 2008;180:5211–5221.
53. Aloia JF, Li-Ng M. Re: epidemic influenza and vitamin D. *Epidemiol Infect*. 2007;135:1095–1098.
54. Grant WB. Variations in vitamin D production could possibly explain the seasonality of childhood respiratory infections in Hawaii. *Pediatr Infect Dis J*. 2008;27:853.

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