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The Impact of Vitamin D on Asthmatic Human Airway Smooth Muscle

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Abstract

Asthma is a chronic heterogeneous disorder, which involves airway inflammation, airway hyperresponsiveness (AHR) and airway remodeling. The airway smooth muscle (ASM) bundle regulates the broncho-motor tone and plays a critical role in AHR as well as orchestrating inflammation. Vitamin D deficiency has been linked to increased severity and exacerbations of symptoms in asthmatic patients. It has been shown to modulate both immune and structural cells, including ASM cells, in inflammatory diseases. Given that current asthma therapies have not been successful in reversing airway remodeling, vitamin D supplementation as a potential therapeutic option has gained a great deal of attention. Here, we highlight the potential immunomodulatory properties of vitamin D in regulating ASM function and airway inflammation in bronchial asthma.

Keywords

Asthma; Airway hyperresponsiveness; airway smooth muscle cells; Immunomodulation; Remodeling; Vitamin D

Introduction

Bronchial asthma is a chronic disorder of the conducting airways, which involves the interplay of both genetic and environmental factors. The hallmarks, including reversible airway obstruction, airway hyperresponsiveness (AHR), cellular infiltration, airway inflammation and airway remodeling, characterize asthma [1,2]. The response in asthma involves the activation of structural cells as well as cells of the innate and adaptive immune systems. Mediators released from this response result in the recruitment of inflammatory

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cells and causes structural changes to the airways, which ultimately result in chronic inflammation [1–3].

Airway smooth muscle (ASM) cells are essential to the integrity and structure of the airways and have long been shown to be the primary cell type responsible for contraction in response to local and circulating factors. Such factors regulate the broncho-motor tone and contribute to AHR, a hallmark feature of asthma [4,5]. There exists some degree of crosstalk between ASM cells and cells of the innate and adaptive immune response. Mediators released from ASM cells have been shown to activate and recruit leukocytes to the airways promoting airway inflammation and AHR [6]. This sets the stage for subsequent chronic inflammation and airway remodeling, which is characterized by structural changes in the airways resulting in ASM hypertrophy and hyperplasia, mucus hypersecretion and sub-epithelial fibrosis (Figure 1) [7].

Current therapeutic options for asthmatic subjects include the use of β_2 -adrenergic receptor agonists, anticholinergic agents and corticosteroids to target airway inflammation and AHR. Targets of β_2 adrenergic receptors include short acting receptor agonist such as albuterol, which provides rapid relief of bronchoconstriction, and long acting receptor agonist, which offers extended control of heightened contractile response to bronchoconstrictors [8]. Corticosteroids are by far the most widely used in the treatment for asthma. These drugs have been successful in controlling asthma symptoms, but are unable to reverse airway remodeling and symptoms persist with prolonged use of these steroids [9]. Anticholinergic agents such as tiotropium, may play a role as add-on therapy for patients with moderate-to-severe asthma [10,11]. However, the safety and usefulness of these drugs in children and the elderly have not been well established [11].

Over the last two decades, Vitamin D has emerged as a potent immunomodulator, regulating both immune and structural cells, including ASM cells. Vitamin D deficiency has been linked to the rise of allergic diseases like asthma with more than 40% of the US population being vitamin D deficient (reviewed in [9] and [12]). Here, we review the role of airway smooth muscle cells in asthma pathogenesis and the potential immunomodulatory effects of the steroid hormone in regulating the function of these cells.

Vitamin D and Asthma

The role of Vitamin D in the pathogenesis of allergic diseases like asthma has gained a great of attention over the last few decades. Vitamin D is a steroid hormone, which originates primarily from the conversion of epidermal 7-dehydro-cholesterol to vitamin D₃ (cholecalciferol) by solar radiation [13]. Cholecalciferol from the skin, as well as that obtained from supplements and dietary sources, is transported to the liver where it undergoes hydroxylation by the enzyme CYP27A1 (25-hydroxylase) to form 25-hydroxyvitamin D₃ [25(OH)D₃]. Further hydroxylation to its active form, 1,25-dihydroxyvitamin D₃, takes place in the kidney and is catalyzed by CYP27B1 (1 α -hydroxylase) [13,14]. Vitamin D signaling occurs through the binding of the active form, calcitriol, to the vitamin D receptor (VDR) in the cytoplasm. The activated VDR forms a heterodimer with the retinoid X receptor, which is then translocated to the nucleus where it can regulate gene expression (Figure 2) [14].

Several studies have highlighted a link between vitamin D deficiency and asthma. Examination of single nucleotide polymorphisms (SNPs) in genes involved with vitamin D metabolism and signaling, particularly CYP24A1 (the main enzyme to degrade 1,25(OH)₂D₃), have shown that there is a heritable component of the vitamin D status in families with asthma [15,16]. Vitamin D deficiency is believed to contribute to asthma pathogenesis by promoting steroid resistance, AHR and airway remodeling [17,18]. A recent meta-analysis study indicated that vitamin D deficiency and insufficiency were associated with an increased risk of childhood asthma [19]. Similar results were found in studies conducted with adolescents and adults [20]. A cross sectional study, including healthy and asthmatic children, reported that serum vitamin D levels were significantly lower in asthmatic children which positively correlated with forced expiratory volume in 1 second (FEV₁) [21].

Vitamin D deficiency was also found to intensify oxidative stress, evidenced by increased reactive oxygen species (ROS) and DNA damage, in peripheral blood mononuclear cells (PMBCs) obtained from patients with severe asthma. This was associated with lower FEV₁ compared to vitamin D sufficient patients [22]. Oxidative stress promotes airway inflammation by inducing the activation of pro-inflammatory genes which result in the release of mediators that enhance airway hyperresponsiveness, ultimately leading to reduced lung function and increased disease severity [23]. It is also believed to play a role in corticosteroid insensitivity by inhibiting the activity of histone deacetylase 2 (HDAC-2), which is crucial in the action of corticosteroids to switch off activated inflammatory genes [24].

Human fetal studies have shown that vitamin D plays a role in growth and development of the fetal lung [25]. In the northeastern United States, low levels of vitamin D intake during pregnancy were associated with increasing wheezing in children by age 3 [26]. A retrospective analysis conducted on adult patients being treated for asthma showed that vitamin D sufficiency was significantly associated with a decrease in total and severe exacerbations as well as decreased emergency room visits in patients with mild to persistent asthma [27]. These studies suggest that vitamin D supplementation may provide some protection against both childhood and adult asthma.

Although lower vitamin D levels have been associated with more severe disease presentation, the results from clinical trials have been inconclusive. The recently concluded VIDA (Vitamin D Add-on Therapy Enhances Corticosteroid Responsiveness in Asthma) clinical trial reported that treatment with vitamin D (100,000 IU once then 4,000 IU/day for 28 weeks orally) did not alter the rate of first treatment failure or exacerbations in patients with persistent asthma and vitamin D insufficiency [28]. Similarly, in a randomized, double-blind, placebo controlled study assessing the effect of oral vitamin D treatment over six weeks, no observable differences were found in IgE levels, eosinophil count or PC₂₀-FEV₁ (a 20% reduction in FEV₁) between placebo and treated groups [29]. This study was, however, performed in a small sample population with only 39 patients, so larger studies will be needed to further explore these effects. Conversely, other studies have highlighted a positive outcome from vitamin D supplementation. Monthly doses of vitamin D, 60,000 IU per month for six months, significantly reduced the number of asthma exacerbations,

requirement for steroids and emergency room visits in asthmatic patients [30]. Vitamin D supplementation was also found to significantly improve FEV₁ in patients with mild to moderate persistent asthma after 24 weeks [31].

It is clear that vitamin D plays a role in airway diseases like asthma since lower levels are often associated with more severe disease presentation and supplementation tends to improve these outcomes. More research is, however, needed to conclusively determine if vitamin D supplementation as an adjunct therapy is a viable option for asthmatic patients.

Airway Smooth Muscle Cells in Asthma Pathogenesis

Airway smooth muscle bundles regulate the broncho-motor tone and are the main effectors of bronchial contraction in the human airways. Contraction of the ASM is mediated by actin-myosin interactions, which are induced by increase in intracellular calcium resulting in activation of the myosin light chain kinase [5,32]. The structural properties of the airway walls are abnormal in asthmatic subjects and these patients tend to have increased smooth muscle mass, which is a characteristic feature of airway remodeling, and is believed to be associated with narrowing of the airways and decreased lung function [5]. Changes in the deposition of extracellular matrix, cellular hypertrophy and hyperplasia contribute to the structurally altered airways and have been shown to be culprits in the severity and exacerbation of asthma [33,34].

Inflammation induced by Airway Smooth Muscle Cells

There is increasing evidence to suggest that the airway smooth muscle is capable of orchestrating inflammation in allergic asthma. Injury to the airway epithelium by allergens and other environmental factors can lead to the release of mediators, which affect airway smooth muscle cells. Studies have shown that the airway epithelium modulates smooth muscle cell proliferation by secreting interleukin-6 (IL-6), IL-8 and monocyte chemoattractant protein-1 (MCP-1) which are known mitogens of ASM cells [35,36]. Human ASM cells have also been shown to secrete MCP-1, which attracts monocytes and lymphocytes and stimulate the release of histamine from basophils, when stimulated with tumor necrosis factor alpha (TNF- α) and IL-1 β [37]. Epithelial damage mediated by house dust mite (HDM) was found to increase proliferation of ASM cells in asthmatic patients. This was later found to be mediated by cysteinyl leukotriene production and over-expression of the CysLTR1 receptor on ASM cells [38].

ASM cells secrete a host of cytokines and chemokines, which have been shown to regulate inflammation [4,8]. Thymic stromal lymphopoietin (TSLP), an IL-17 like pro-allergic cytokine, has been shown to promote the development of T-helper 2 (Th2) immune responses in allergic asthma. TSLP expression was found to be elevated in ASM bundles of patients with mild to moderate disease [39]. TSLP has also been shown to induce human airway smooth muscle (HASM) cell migration through signal transducer and activation of transcription 3 (STAT-3) as well as actin polymerization and cell polarization [40]. Activation of the TSLP receptor on HASM cells led to enhanced secretion of IL-6, IL-8/CXCL8 and eotaxin/CCL11 [41]. These mediators are well known for the roles in promoting

mucus hypersecretion (IL-6), recruiting inflammatory cells to the airways (IL-8 and CCL11) as well as promoting differentiation of lymphocytes (IL-6) [42].

Studies using ASM cells from asthmatic and non-asthmatic patients reported that Th17 associated cytokines, namely IL-17A, IL-17F and IL-22, were able to promote proliferation and survival of these cells. This induced proliferation was through the extracellular signal regulated protein kinases-1 and -2 (ERK1/2) mitogen activated protein kinases (MAPK) and NF κ B pathways [43]. IL-17 also promoted migration of ASM cells [44] as well as significant increases in the production of eotaxin, which was reduced by a neutralizing anti-IL-17A antibody [45].

In the airways, the major antibody responsible for promoting inflammation is immunoglobulin E (IgE). Its action is mediated primarily by binding to high affinity (Fc ϵ RI) IgE receptors on mast cells and basophils [1,2]. Airway smooth muscle cells have been shown to express tetrameric Fc ϵ RI receptors, which upon activation led to increased intracellular calcium as well as enhanced release of pro-inflammatory mediators such as IL-4, IL-5, IL-13 and eotaxin/CCL11 [46]. A later study revealed that the incubation of human ASM cells with TNF- α , IL-1 β or IL-4 led to significant upregulation of the Fc ϵ RI α -chain mRNA and protein expression [47]. IgE was also shown to induce HASM cell proliferation by activation of the ERK1/2, p38, c-Jun N-terminal kinase (JNK) MAPK and Akt kinases [48]. Collectively, these results suggest that ASM cells may contribute to inflammation by upregulating Th2 cytokines as well as by activation of the IgE/Fc ϵ RI complex to induce cell proliferation.

Interaction between Airway Smooth Muscle and Immune Cells

The airway smooth muscle bundle also expresses a number of cell adhesion molecules which play a role in the recruitment and retention of inflammatory cells in the airways perpetuating airway inflammation [49,50]. Human ASM was shown to maintain mast cell survival *in vitro* and induce mast cell proliferation via cell-cell contact through interaction between membrane bound stem cell factor (SCF) on ASM and soluble IL-6 and cell adhesion molecule-1 (CADM-1) expressed on mast cells [51]. Localization of mast cells within the airway smooth muscle bundle plays a critical role in the development of AHR. Mast cells secrete cytokines, which have direct effects on ASM proliferation and contraction. Analysis of bronchial biopsies of patients with and without asthma revealed that IL-33-stimulated mast cells promoted ASM repair and indirectly induced ASM contractions [52]. In a recent study, mast cells were shown to be directly activated by ASM chemokines, CXCL10 and CCL5. Both chemokines were able to induce mast cell degranulation [53].

There exists some degree of crosstalk between the airway smooth muscle bundle and T-cells. Human ASM cells express CD44 and have been shown to upregulate intracellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) expression when exposed to TNF- α . Activated T-cells were found to adhere to the ASM through the interaction of lymphocyte function-associated antigen 1 (LFA-1) and very late antigen 4 (VLA-4) on T-cells with CD44, VCAM-1 and ICAM-1 on ASM cells [54–56]. In more recent studies, T-cells were found to adhere to the ASM bundle by VCAM-1, and induce proliferation of ASM cells by direct contact with smooth muscle alpha-actin (α -SMA+) cells

in asthmatic patients [57]. It has been recently shown that direct contact between ASM cells and T-cells facilitate the transfer of myeloid leukemia cell differentiation protein (Mcl-1) via nanotubes, promoting T-cell survival [58].

The role of the airway smooth muscle in asthma pathogenesis has been extensively reviewed [4–6,8,59]. Collectively, these results highlight that airway smooth muscle cells not only control contraction of the airways and promote AHR but can also orchestrate inflammation by cross talk with inflammatory cells as well as promoting the release of potent mediators, which contribute to underlying pathophysiology of allergic asthma (Figure 1). Given these facts, the development of strategies that specifically target ASM cells will be crucial for treating abnormalities of the ASM bundle in asthmatic patients.

Vitamin D and Airway Smooth Muscle Cell Function

Increased smooth muscle mass is a key feature of structural changes in the airways which contributes to narrowing of the lumen and hypercontractility in patients with asthma [60]. Although current asthma therapies control the symptoms of the disorder, there is little evidence to suggest that the current therapies affect airway remodeling [61]. Given that lower levels of serum vitamin D has been shown to be associated with increased ASM mass and AHR, worse control of asthma and increased exacerbations in children, adolescents and adults [18,20–22], vitamin D could be a potent modulator of ASM cells (Figure 2).

Proliferation and Migration of Airway Smooth Muscle Cells

Examination of human airway smooth cells *in vitro* demonstrated the expression of functionally active vitamin D receptors. It was found that when these cells were stimulated with 1- α , 25-dihydroxy vitamin D₃ (calcitriol), there was increased expression of 24-hydroxylase (CYP24A1) as well as genes involved in cell growth, morphogenesis and survival [62]. Vitamin D has also been shown to regulate transcription of genes involved in airway remodeling, thus reducing and/or ameliorating hallmark features of remodeling in several studies using human ASM cells. Treatment of HASM cells, passively sensitized with asthmatic serum, with 1,25(OH)₂D₃ suppressed proliferation, proliferating cell nuclear antigen (PCNA) expression as well as transition from G1 to S phase of the ASM cell cycle. The 1,25(OH)₂D₃ was also able to downregulate the protein and mRNA expression of matrix metalloproteinase-9 (MMP-9) and a disintegrin metalloprotease-33 (ADAM-33), both of which play a role in airway remodeling and increased disease severity associated with allergic asthma [63]. The vitamin D₃ metabolite was also able to inhibit proliferation of fetal ASM cells induced by TNF- α and TGF- β via the ERK-mediated signaling pathway [64]. Treatment of human ASM cells with calcitriol inhibited platelet-derived growth factor (PDGF)-induced ASM growth by inhibiting phosphorylation of retinoblastoma protein (Rb) and checkpoint kinase 1 (Chk1). Vitamin D, therefore, seems to exert its anti-proliferative effects by modulating airway smooth muscle cell cycle [17].

Extracellular matrix deposition and epithelial-mesenchymal transition

Analysis of the airway smooth muscle transcriptome in fatal and non-asthmatic derived ASM cells revealed that vitamin D induced differential expression of functional genes, such

as those involved with the extracellular matrix and response to steroid hormone stimuli and wounding, as well as genes involved in cytokine and chemokine activity [65]. Using human fetal ASM cells as a model of the early post natal airways, it was found that calcitriol attenuated TNF- α enhancement of MMP-9 expression and activity as well as TNF- α and TGF- β induced collagen II expression and deposition [64]. These mediators are known to contribute to epithelial-mesenchymal transition (EMT) and airway remodeling [66]. We have recently reported the inhibitory effect of calcitriol on the expression of EMT markers, including snail, vimentin and N-cadherin, in TGF- β 1-treated airway epithelial cells [67]. In addition, calcitriol inhibited the effect of TGF- β 1 to decrease E-cadherin in airway epithelial cells and decreased cell motility [67]. Since airway epithelial cells closely interact with airway smooth muscle cells, these findings further highlight the beneficial effect of vitamin D in regulating EMT, and thus, sub-epithelial fibrosis and airway remodeling.

Cytokine Secretion by Airway Smooth Muscle Cells

Previously highlighted studies have shown that the airway smooth muscle is capable of secreting a host of mediators that help to orchestrate inflammation. Secretion of RANTES (regulated upon activation, normal T-cell expressed and secreted), which is chemotactic for T-cells, eosinophils and basophils, was partially inhibited by treatment with calcitriol in human ASM cultures stimulated with TNF- α . In cells treated with TNF- α or IFN- γ , calcitriol, in conjunction with the corticosteroid fluticasone, was able to inhibit RANTES secretion by 60%. Calcitriol was also found to significantly decrease fractalkine (FKN)/CX3CL1 levels which was further enhanced by steroid treatment. Results highlight a potential role for vitamin D in the treatment of steroid-resistant asthma [68]. Treatment of ASM cells derived from fatal asthmatics with vitamin D inhibited TNF- α -induced IL-8 secretion from these cells when compared to non-asthmatic cells even though the fatal asthmatic ASM cells had significantly higher baseline levels of IL-8 [65].

Activation of NF- κ B has been shown to induce transcription of inflammatory mediators involved in allergic asthma [69]. The 1,25(OH) $_2$ D $_3$ was reported to reduce the DNA binding capacity of NF- κ B and inhibit translocation of the NF- κ B p65 complex to the nucleus in human ASM cells passively sensitized with asthmatic serum [70]. Calcitriol was also found to significantly decrease mRNA and protein expression of importin- α 3 and NF- κ B p65 (RelA) [62]. This decreased activation was confirmed by decrease in the release of RelA inducible molecules such as IL-5, IL-6 and IL-8 [71]. These results suggest that vitamin D may attenuate airway remodeling by an inhibition of NF κ B mediated signaling thus decreasing the secretion of pro-inflammatory cytokines.

Expert Commentary

The contribution of the airway smooth muscle in the pathogenesis of asthma has been highlighted by several studies. Over the last few decades, we have made remarkable advances in understanding of the role and mechanism of vitamin D as an anti-inflammatory mediator. It is now clear that Vitamin D may influence remodeling and inflammation orchestrated by ASM cells by regulating the growth, proliferation and contraction of these cells. Several of these studies, however, have relied heavily on *in vitro* experiments and

although these experiments have been useful in understanding the mechanisms that lead to abnormalities in the ASM bundle and how vitamin D affects these cells, culture conditions represent a controlled environment which may be much different than in the human body.

Although the relationship between asthma and vitamin D has been well characterized by *in vitro* studies as well as studies using animal models, clinical trials investigating the effects of vitamin D supplementation as a potential therapeutic option have not provided clear answers. This inconsistency has, in part, been due to variations in the measurement of vitamin D levels (serum versus recorded intake from food) as well as the source of vitamin D being examined (food versus direct sunlight) [61]. Clearly, further studies are needed in this area to provide concrete evidence regarding the use of vitamin D as a potential adjunct in the treatment option for asthma.

Five Year View

As highlighted in this review, several studies suggest that vitamin D supplementation could decrease exacerbations of asthma; however, results from clinical trials to-date have produced conflicting evidence. Over the next five years, it would be paramount to conduct targeted clinical trials with representative sample sizes to determine the mechanisms underlying vitamin D modulation of ASM and by extension airway remodeling and inflammation in asthma. Trials should be designed based on current knowledge of pathways involved in the immunomodulation by vitamin D. Studies are also needed to decipher what is the optimal level of vitamin D for different age groups, gender, ethnicity and asthmatic phenotypes. In addition to determining the optimal vitamin D doses to be given for therapeutic intervention, the most effective route of administration also needs to be addressed. Results from these trials will no doubt provide more concrete evidence as to whether vitamin D supplementation should be mainstay treatment for airway remodeling, used in conjunction with current therapeutic options or be abandoned as another failed attempt in asthma therapy.

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Key Issues

- Airway smooth muscle bundles regulate the broncho-motor tone and are the main effectors of bronchial contraction in human airways. They are also able to orchestrate inflammation leading to airway hyperresponsiveness and remodeling.
- The airway smooth muscle bundle is aberrant in asthmatic patients and current therapeutic options are not effective at altering airway remodeling.
- Vitamin D deficiency has been associated with increased airway inflammation and exacerbations in asthmatic patients.
- ASM cells express a functionally active vitamin D receptor
- Vitamin D has been shown to decrease ASM proliferation, migration as well as cytokine secretion *in vitro*.
- Though there is a clear link between vitamin D status and airway inflammation in asthma, results from clinical trials are inconclusive.
- Targeted clinical trials are needed to conclusively decipher whether or not vitamin D supplementation is a viable treatment option for asthmatics.

Figure 1. Airway smooth muscle cells in the pathogenesis of asthma

Injury to the airway epithelium, by allergens or environmental factors, lead to the release of mediators that contribute to smooth muscle cell proliferation. Activation of ASM cells by TSLP promotes increased smooth muscle contraction as well as the release of CCL11, IL-6 and IL-8 which recruit inflammatory cells. ASM cells also secrete TSLP which is believed to act in a paracrine manner. ASM cells also express cell adhesion molecules such as ICAM-1 and VCAM-1 as well as SCF which enables adhesion of mast cells and T-cells to ASM bundle. Mast cells, under the influence of ASM chemokines, undergo degranulation releasing leukotrienes and prostaglandins. These mediators contribute to ASM contraction and ECM deposition which can in turn contribute to airway hyperresponsiveness and airway remodeling in asthma. DC, dendritic cell; Th, T helper; MC, mast cell; TSLP, thymic stromal lymphopoietin; MCP, monocyte chemotactic protein; CCL, chemokine ligand; CXCL, C-X-chemokine; ECM, extra cellular matrix; SCF, stem cell factor; CADM, cell adhesion molecule; ICAM, intracellular adhesion molecule; VCAM, vascular cell adhesion molecule; LFA, lymphocyte function-associated antigen; VLA, very late antigen

Figure 2. Immunomodulatory effects of vitamin D on airway smooth muscle cell
 Vitamin D from the sunlight or the diet is converted to 25(OH)D₃, the main circulating form, by CYP27A1. It is further hydrolyzed to its active form, 1,25(OH)₂D₃ by CYP27B1 which enables binding of the active form to the vitamin D receptor in the cytoplasm. The VDR associates with RXR and is translocated to the nucleus where it can regulate gene expression. In ASM cells, vitamin D has been shown inhibit the progression of the cell cycle thus decreasing ASM proliferation; decrease the production of potent inflammatory mediators such as TNF-α, IL-8 and RANTES which result in reduced chemotaxis of inflammatory cells to the airways. Vitamin D has also been shown to modulate the expression of mediators involved in collagen deposition and airway remodeling. The overall effect of this modulation is decreased inflammation, airway hyperresponsiveness and airway remodeling associated with asthma. VDR, vitamin D receptor; RXR, retinoid X receptor; VDRE, vitamin D response element; ASM, airway smooth muscle; MMP, matrix metalloproteinase; ADAM, a disintegrin metalloprotease; TNF, tumor necrosis factor; TGF, transforming growth factor; RANTES, regulated upon activation, normal T-cell expressed and secreted.