

AIRWAY REMODELING

1,25-Dihydroxyvitamin D₃ (1,25-(OH)₂D₃) Attenuates Airway Remodeling in a Murine Model of Chronic Asthma

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Objectives. 1,25-Dihydroxyvitamin D₃ (1,25-(OH)₂D₃) has immune- and inflammation-modulating properties in asthma, but its possible effects on asthmatic airway remodeling remain uncertain. In this study, we investigated the effects of 1,25-(OH)₂D₃ on airway remodeling in a murine model of chronic asthma and investigated its role in regulating nuclear factor-κB (NF-κB) activation. **Methods.** BALB/c mice were sensitized to ovalbumin (OVA) and subsequently exposed to intranasal OVA challenges for 9 weeks. Some mice also received an intraperitoneal injection of 1,25-(OH)₂D₃ at the time of challenge. At the end of the challenge period, mice were evaluated for chronic airway inflammation and airway remodeling. Nuclear translocation of NF-κB p65 in lung tissue was examined by Western blot. Inhibitor of NF-κB alpha (IκBα) expression was determined by real-time quantitative Reverse Transcription Polymerase Chain Reaction (RT-PCR) and Western blot. Phosphorylated IκBα protein expression was also determined by Western blot. **Results.** 1,25-(OH)₂D₃ treatment reduced OVA-induced chronic inflammation in lung tissue and attenuated established structural changes of the airways, including subepithelial collagen deposition, goblet cell hyperplasia, and increased airway smooth muscle mass. 1,25-(OH)₂D₃ also inhibited the nuclear translocation of NF-κB p65 in lung tissue. Concurrently, 1,25-(OH)₂D₃ induced increased IκBα protein levels via inducing increased IκBα mRNA levels and decreased IκBα phosphorylation. **Conclusion.** 1,25-(OH)₂D₃ could attenuate asthmatic airway remodeling and its inhibition of NF-κB activation may underlie this protective effect.

Keywords airway remodeling, asthma, 1,25-dihydroxyvitamin D₃, IκBα, nuclear factor kappa B

INTRODUCTION

Airway remodeling is an important component of asthma that leads to irreversible airway obstruction and persistent lung function decline (1). This remodeling is characterized by structural and morphometric changes of the airways, including subepithelial fibrosis, goblet cell (GC) hyperplasia, and airway smooth muscle (ASM) hyperplasia/hypertrophy, which are associated with increased asthma severity (2). Although glucocorticoids remain the first-line agents for treatment in current asthma guidelines, they have a limited ability to reverse or prevent the progression of airway remodeling (3). Thus, there is a need for alternative approaches to prevent airway remodeling.

The steroid hormone 1,25-dihydroxyvitamin D₃ (1,25-(OH)₂D₃) is the active metabolite of vitamin D. Upon binding to a specific nuclear vitamin D receptor (VDR), it modulates various biological activities. Although 1,25-(OH)₂D₃ is currently occasionally administered to severe asthmatic patients as part of a prophylactic regimen against glucocorticoid-induced osteoporosis, considerable effort has been devoted to exploring its immune- and inflammation-modulating properties in asthma (4, 5). However, to date, few data are available regarding the

possible effects of 1,25-(OH)₂D₃ on asthmatic airway remodeling.

We previously showed that 1,25-(OH)₂D₃ could directly inhibit the effects of asthmatic serum on the proliferation of human airway smooth muscle cells (HASMCS), the most important structural cell involved in airway remodeling, and their expression of matrix metalloproteinase-9 (6). Other studies have shown that VDR deletion upregulated matrix metalloproteinases in the lung, which are critically involved in the airway remodeling process (7). Further, vitamin D may inhibit the profibrotic effects of transforming growth factor β on lung fibroblasts and epithelial cells and blunt the epithelial-to-mesenchymal transition as part of its anti-remodeling effects (8). These findings suggest the possibility that 1,25-(OH)₂D₃ could have an effect on airway remodeling. Thus, in this study we investigated the effects of 1,25-(OH)₂D₃ on airway remodeling in a murine model of chronic asthma.

Nuclear factor kappa B (NF-κB) is a transcription factor with multiple regulatory roles. When cells are at rest, NF-κB binds to its inhibitor IκB [among which inhibitor of NF-κB alpha (IκBα) is most closely associated with NF-κB activation] and forms an inactive trimer (p50/p65/IκB) in the cytoplasm. When cells are stimulated, such as by tumor necrosis factor (TNF) or Phorbol-12-myristate-13-acetate (PMA), IκB is removed from this trimer, which exposes the p50 subunit's translocation signal and the p65 subunit's DNA-binding site. Subsequently, the p50/p65 dimer translocates from the cytoplasm into the nucleus and specifically

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binds to NF- κ B target genes, which then exert their regulatory effects involving the production of inflammatory cytokines and chemokines, proliferation and apoptosis of cells, and cross-linking of extracellular matrix components. Based on this mechanism, NF- κ B activation requires (i) dissociation of inhibitor I κ B and (ii) nuclear translocation of NF- κ B.

Numerous studies have confirmed that NF- κ B is over-activated in asthma patients (9). As one of the attractive signaling pathways in the pathogenesis of asthma, NF- κ B can act at different levels of airway remodeling, such as regulating extracellular matrix remodeling, ASM cell phenotypes, and the synthesis of collagen (10). However, whether the NF- κ B signaling pathway can be regulated by 1,25-(OH) $_2$ D $_3$ to control airway remodeling is not clear. Thus, we also investigated the mechanism underlying 1,25-(OH) $_2$ D $_3$ for regulating NF- κ B activation in a murine model of chronic asthma. Our findings should provide a theoretical basis for the clinical treatment of asthma with 1,25-(OH) $_2$ D $_3$.

METHODS

Reagents

Ovalbumin (OVA), aluminum hydroxide, and 1,25-(OH) $_2$ D $_3$ were purchased from Sigma-Aldrich Int. (Louis, MO, USA). A polyclonal antibody against α -smooth muscle actin (α -SMA) was purchased from Boster Biotechnology Co. (Wuhan, Hubei, China). Polyclonal antibodies against NF- κ B p65, I κ B α , and phosphoserine-I κ B α (phospho-I κ B α) were purchased from Santa Cruz Biotechnology Co. (Santa Cruz, CA, USA). NE-PER nuclear and cytoplasmic extraction reagents kit was purchased from Pierce Biotechnology Inc. (Rockford, IL, USA).

Mice

Male BALB/c mice (weight: 17–21 g) were purchased from the Laboratory Animal Research Center of the Fourth Military Medical University (Xi'an, PR China) and used at 8–10 weeks of age. All animal experimental protocols were approved by the Animal Subjects Committee of the Fourth Military Medical University (Xi'an, PR China).

OVA Sensitization and Challenge

BALB/c mice were immunized by intraperitoneal (i.p.) injection of 50 μ g of OVA complexed with 5 mg of aluminum hydroxide on days 0, 7, and 14, followed by OVA challenges (nebulized 2.5% solution; 30 min/d) 3 times weekly for 9 weeks. At the time of a challenge, three groups of mice were given different treatments (6 mice/group): (i) mice in an asthma group were injected i.p. with 300 μ L phosphate- buffer saline (PBS) containing 0.9% ethanol at 30 min before each inhalation; (ii) mice in a 1,25-(OH) $_2$ D $_3$ group (VD group) were injected i.p. with 100 ng 1,25-(OH) $_2$ D $_3$ dissolved in ethanol and further diluted with 300 μ L PBS as previously described to a final concentration of 0.9% at the same time (11); (iii) mice in a control group were sensitized, but were not challenged with OVA during the study; these mice also received i.p injection with 300 μ L PBS containing 0.9%

ethanol on the same schedule as the mice with OVA challenges. At 24 h after the last challenge, the mice were humanely killed and lung samples were harvested.

Lung Histopathology

At the time of killing, left lung samples were prepared for histology by formalin fixation and paraffin embedding. Sections (4 μ m thick) were prepared for staining by deparaffinization and hydration. Hematoxylin and eosin (HE) staining was used to assess chronic airway inflammation. Alcian blue-periodic acid Schiff (AB-PAS) staining was used to enumerate GCs. Masson trichrome staining was used to detect subepithelial collagen thickness.

Morphometric Analysis of Airway Inflammation and Structural Changes

At least 10 bronchioles measuring 150–250 μ m in luminal diameter were analyzed on each section. The area of the mucous cell layer (the epithelial layer), the area of the smooth muscle mass, and collagen area were measured by image analysis using Image Pro-Discovery software (Media Cybernetics). These areas were standardized to the perimeter of the basement membrane (Pbm) to account for variations in bronchiole diameters.

Lung sections were also scored blindly on a scale of 0–4 for inflammation and epithelial thickening as described previously (12, 13). Inflammation scores were as follows: 0 = no inflammation; 1 = inflammatory cells present; 2 = multiple loci of inflammation; 3 = many inflammatory cells around bronchi; and 4 = inflammatory cells throughout the lung. Epithelial thickening scores were as follows: 0 = normal; 1 = some epithelial thickening; 2 = multiple loci of thickened airway; 3 = airways nearly completely obstructed by epithelial thickening; and 4 = normal structure not present. In addition, GCs were counted in AB-PAS-stained sections and expressed as the number of cells per 100 μ m Pbm.

Immunohistochemistry for α -SMA Expression in Airways

Lung sections were stained using standard immunochemical technique. An anti- α -SMA primary antibody was used at a dilution of 1:500. Species- and isotype-matched antibodies were used as controls in place of the primary antibody. The area of peribronchial α -SMA staining in paraffin-embedded lung sections was outlined and quantified by image analysis as described above.

Western Blot Analysis

The proteins (including total, nuclear, and cytoplasmic proteins) in lung tissue were extracted using NE-PER nuclear and cytoplasmic extraction reagent kit according to the manufacturer's instructions. The total protein in lung tissue was subjected to electrophoresis using 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane. The primary antibodies were polyclonal antibodies against NF- κ B p65, I κ B α , and phospho-I κ B α used at a 1/1000 dilution each. Immunoreactive bands were visualized using an Enhanced Chemiluminescence (ECL) kit. Band intensities were determined using a gel analysis system and

quantitatively analyzed by normalizing to the band intensity for β -actin. The protein levels of NF- κ B p65 in the cytoplasm and nucleus were also determined by the same method using an anti-NF- κ B p65 antibody.

Real-Time Quantitative RT-PCR

Total RNA was extracted from lung tissue using TRIzol reagent and then reverse transcribed to cDNA, which was used for real-time quantitative RT-PCR analysis. PCR amplification was done using a FastStart DNA Master SYBR Green I mixture. PCR conditions for I κ B α were 30 cycles of denaturation at 94°C for 45 s, annealing at 60°C for 1 min, and extension at 72°C for 90 s. Glyceraldehyde-3-phosphatedehydrogenase (GAPDH) cDNA samples were amplified with 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 2 min. Target gene mRNA levels were normalized to the mRNA level of the GAPDH housekeeping gene. Oligonucleotide primers used for I κ B α and GAPDH mRNA are shown in Table 1.

Statistical Analysis

The results are expressed as means $\pm$ SDs. All data were tested for variance homogeneity. Groups were compared using one-way analysis of variance or nonparametric

Kruskal–Wallis test. Multiple comparisons between groups were made by Student–Newman–Keuls test or a Nemenyi test. Statistical significance was set at a p -value of $<.05$.

RESULTS

1,25-(OH)₂D₃ Inhibits the Asthma-Associated Inflammatory Response

As compared with control mice (Figure 1A), the OVA-challenged mice developed obvious increases in airway tissue epithelial thickness and in the numbers of peribronchial inflammatory cells, which were primarily eosinophils but often also included neutrophils and macrophages (Figure 1B). However, 1,25-(OH)₂D₃ treatment markedly reduced these increases in the challenged airways (Figure 1C). Thus, the inflammation scores and the epithelial thickening scores in the VD group were lower compared to those in the asthma group ($p = .007$) (Figure 1D and 1E).

1,25-(OH)₂D₃ Inhibits GC Hyperplasia

Only a relatively few, isolated GCs could be observed in the airway epithelium of control mice (Figure 2A). OVA challenge resulted in a readily observable increase in the amount of airway GCs (Figure 2B), which was inhibited by 1,25-(OH)₂D₃ treatment (Figure 2C). Counting the numbers of GCs confirmed the observations made by visual inspection of the slides (Figure 2D).

1,25-(OH)₂D₃ Attenuates Other Structural Changes of the Airway in Chronic Asthma

Morphometric measurements made for HE-stained lung sections (Table 2) showed that there was an obvious inhibition in the thickness of the collagen layer in 1,25-(OH)₂D₃-treated and OVA-challenged mice relative to the OVA-challenged

TABLE 1.—List of primers used for RT-PCR cDNA amplification.

Gene	Primer sequence	Product size (bp)
I κ B α	F: 5'-CCGCACAGCCATGTTTCAG-3'	125
	R: 5'-CATGGAGTCCAGGCCGCTGTCGTG-3'	
GAPDH	F: 5'-AACGACCCCTTCATTGAC-3'	191
	R: 5'-TCCACGACATACTCAGCAC-3'	

Note: F, forward primer; R, reverse primer.

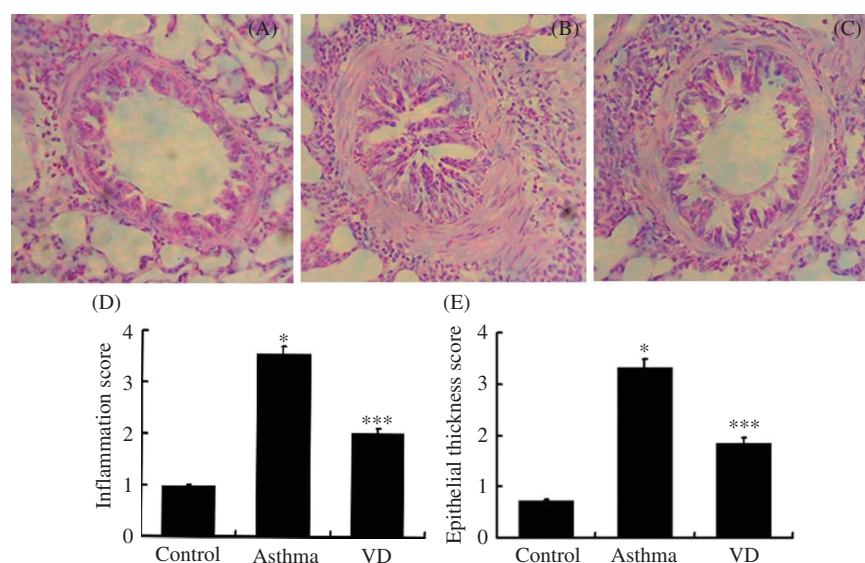


FIGURE 1.—1,25-(OH)₂D₃ prevents an airway inflammatory response in a murine model of chronic asthma. Lungs from each group of mice were fixed, sectioned, and stained with HE. The photomicrographs are representative of lungs removed from mice in (A) control group, (B) asthma group, and (C) VD group. All images were taken at 200 $\times$ magnification. Peribronchial inflammation was quantified by blinded assessments of (D) inflammation scores and (E) epithelial thickening scores for the different groups of mice as described in the section “Methods”. The results are expressed as means $\pm$ SDs ($n = 6$ for all groups; * $p < .05$ vs. control group, ** $p < .05$ vs. asthma group).

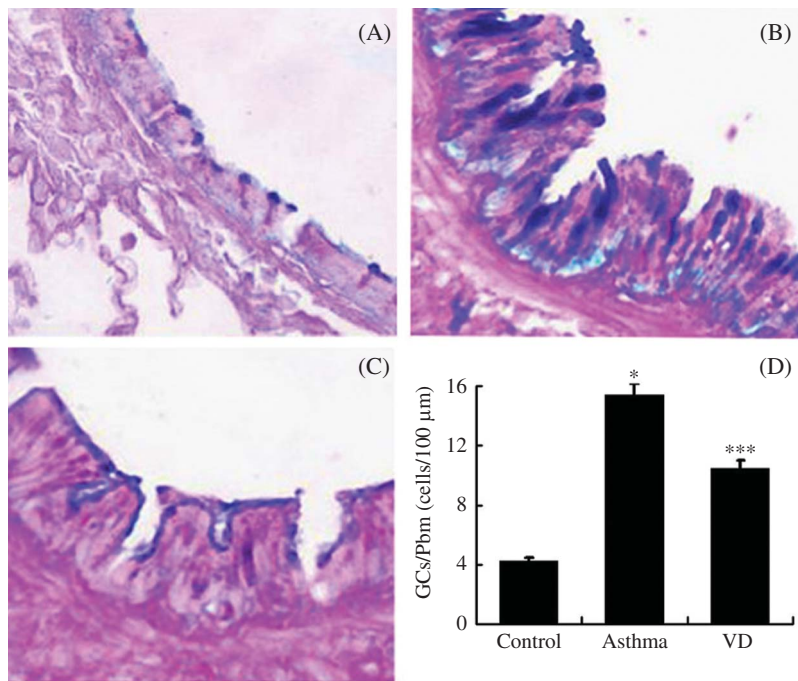


FIGURE 2.—1,25-(OH)₂D₃ prevents airway GC hyperplasia in a murine model of chronic asthma. Lungs from each group of mice were fixed, sectioned, and stained with AB-PAS. The photomicrographs are representative of lungs removed from mice in (A) control group, (B) asthma group, and (C) VD group. All images were taken at 400× magnification. The amount of GCs was quantitated in lung sections (D) standardized to the Pbm to account for variations in bronchiole diameters. The results are expressed as means ± SDs ($n = 6$ for all groups; * $p < .05$ vs. control group, *** $p < .05$ vs. asthma group).

TABLE 2.—Morphometric analysis of airway wall thickness.

Group	WAmuc/Pbm ($\mu\text{m}^2/\mu\text{m}$)	Wcol/Pbm ($\mu\text{m}^2/\mu\text{m}$)	WAm/Pbm ($\mu\text{m}^2/\mu\text{m}$)
Control	7.14 ± 0.51	8.37 ± 0.87	2.43 ± 0.51
Asthma	21.57 ± 0.74*	16.73 ± 1.41*	7.18 ± 1.13*
VD	15.33 ± 0.37***	10.75 ± 1.14***	4.35 ± 0.97***

Notes: At least 10 bronchioles measuring 150–250 μm in luminal diameter were analyzed on each section. The area of the mucous cell layer (WAmuc), the area of the smooth muscle mass (WAm), and the collagen area (Wcol) were measured by image analysis using Image Pro-Discovery software. All data were standardized to the Pbm to account for variations in bronchiole diameters. The results are expressed as means ± SDs ($n = 6$ per group; * $p < .05$ vs. control group, *** $p < .05$ vs. asthma group).

mice without this treatment ($p = .019$). Similarly, but less strikingly, these results were also seen for the thickness of the mucous cell layer of the epithelium and the ASM mass.

In addition, OVA challenge resulted in increased α -SMA staining (Figure 3B) on the lung sections compared to sections from control mice (Figure 3A). Treatment with 1,25-(OH)₂D₃ reduced this effect (Figure 3C). Quantifying the α -SMA-stained areas (Figure 3D) confirmed the observations made by visual inspection (Figure 3A–3C).

1,25-(OH)₂D₃ Inhibits the Nuclear Translocation of NF- κ B p65

Western blot analysis showed that OVA-challenged mice had increased NF- κ B p65 levels in the total protein in lung tissue compared to control mice. However, 1,25-(OH)₂D₃ treatment reduced these levels induced by OVA challenge ($p = .017$) (Figure 4A and 4B). Furthermore, OVA challenge increased the NF- κ B p65 level in the nucleus and correspondingly decreased its

cytoplasmic level; that is, OVA challenge induced NF- κ B p65 translocation to the nuclear compartment. However, 1,25-(OH)₂D₃ markedly attenuated this nuclear translocation induced by OVA challenge ($p < .001$) (Figure 4A and 4C).

1,25-(OH)₂D₃ Increases the I κ B α Level

We next investigated whether the decrease in NF- κ B activity was associated with its inhibitor I κ B α . As shown in Figure 5A and 5B, 1,25-(OH)₂D₃ treatment effectively increased the I κ B α protein level in lung tissues of OVA-challenged mice ($p = .008$). Similar results were found for the I κ B α mRNA levels ($p = .012$; Figure 6), which we speculated was one reason for the increased expression of I κ B α protein due to 1,25-(OH)₂D₃.

1,25-(OH)₂D₃ Decreases I κ B α Phosphorylation

I κ B α protein degradation is another possible reason for an effect on I κ B α protein levels. Phosphorylation is the first step prior to I κ B α ubiquitination and degradation (14). Thus, we examined the effect of 1,25-(OH)₂D₃ on I κ B α phosphorylation by Western blot analysis. Figure 5A shows that chronic OVA challenge caused a notable increase in phosphorylation of the I κ B α protein, while 1,25-(OH)₂D₃ decreased this phosphorylation. Because the phospho-I κ B α level could depend on the total I κ B α level, the phospho-I κ B α level was calculated from the ratio of phospho-I κ B α /total I κ B α to compensate for differences in the amounts of total I κ B α . As expected, quantifying the phospho-I κ B α protein level (Figure 5C) confirmed the observations made by visual inspection (Figure 5A).

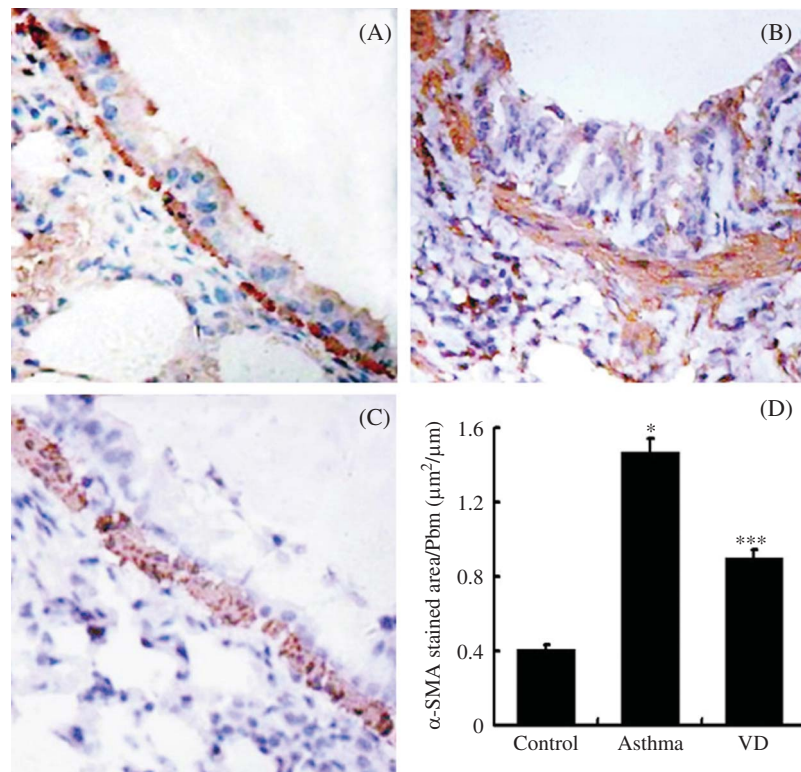


FIGURE 3.—1,25-(OH)₂D₃ prevents the increase in airway α-SMA-immunostained areas in a murine model of chronic asthma. Lungs from each group of mice were fixed, sectioned, and immunostained with α-SMA. The photomicrographs are representative of lungs removed from mice in (A) control group, (B) asthma group, and (C) VD group. All images were taken at 400× magnification. The areas of peribronchial α-SMA immunostaining were quantified in lung sections (D) standardized to the Pbm to account for variations in bronchiole diameters. The results are expressed as means ± SDs ($n = 6$ for all groups; * $p < .05$ vs. control group, *** $p < .05$ vs. asthma group).

DISCUSSION

The results of this study demonstrated for the first time that following chronic OVA challenge, 1,25-(OH)₂D₃ treatment not only efficiently reduced inflammatory cell infiltration in lung tissue, but also attenuated established structural changes of the airways. These results highlight the potential of 1,25-(OH)₂D₃ as an anti-remodeling therapy for asthma.

Subepithelial fibrosis is a key feature of airway remodeling in asthma and its development can drive remodeling processes (15). It was reported previously that subepithelial fibrosis only developed in a murine model after 4–6 weeks of allergen exposure (16). Thus, we administered inhalational allergen for 9 weeks in our study. In keeping with previous studies that showed that 1,25-(OH)₂D₃ could protect against fibrosis in various animal models (17, 18), we also showed that 1,25-(OH)₂D₃ treatment could efficiently inhibit subepithelial collagen deposition induced by repeated OVA exposures.

In addition to collagen deposition, epithelial changes, particularly GC hyperplasia, are also observed in chronic asthma. As a prominent feature of even mild asthma, GC hyperplasia in the airway epithelium promotes excessive mucin secretion (19). In this study, 1,25-(OH)₂D₃ obviously reduced epithelial thickening and prevented GC hyperplasia in a chronic asthma model, which

provided a link between 1,25-(OH)₂D₃ and mucin production for the first time.

Although airway remodeling involves alterations of several cell types, airway smooth muscle cells (ASMCs) have recently received considerable attention. Increased ASM mass is regarded as a key feature of and the most plausible explanation for the chronic disease process (20–22). By image analysis and α-SMA immunohistochemistry, we demonstrated that 1,25-(OH)₂D₃ reduced the thickness of the ASM layer in OVA-challenged mice. However, it remains uncertain whether ASMCs were directly regulated by 1,25-(OH)₂D₃ or whether ASMC changes resulted from some other downstream events regulated by airway fibrosis itself. In support of the former possibility, our previous study and other studies showed that 1,25-(OH)₂D₃ had direct anti-proliferative effects on HASMCs *in vitro* (6, 23, 24). Of course, the present results showing 1,25-(OH)₂D₃-reduced airway fibrosis might further contribute to the changes in ASMCs by 1,25-(OH)₂D₃.

Taken together, the results of this study showed that 1,25-(OH)₂D₃ could negatively regulate asthmatic airway remodeling involving many different pathways. There is a well-known relationship between airway remodeling and lung function (25, 26). Thus, the inhibition of airway remodeling by 1,25-(OH)₂D₃ that was clearly demonstrated in this study could provide an explanation for

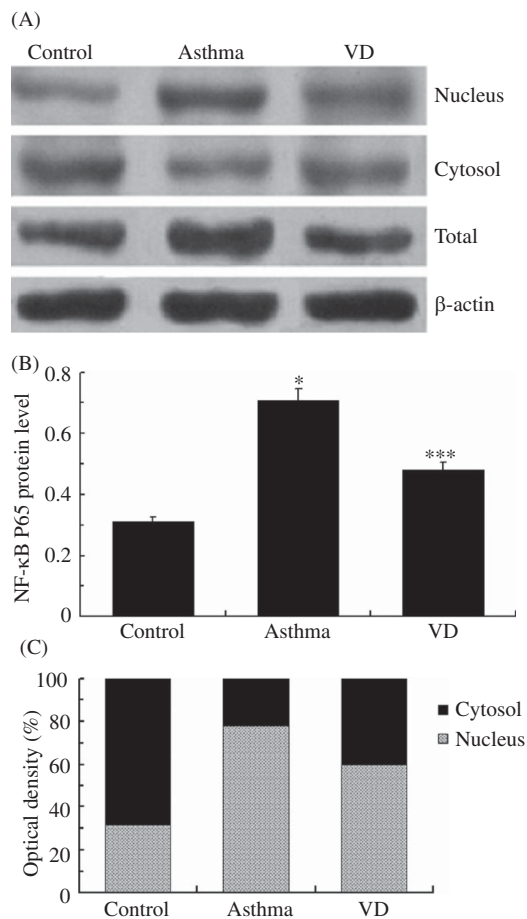


FIGURE 4.—1,25-(OH)₂D₃ prevents the nuclear translocation of NF-κB p65 in a murine model of chronic asthma. The proteins (including total, nuclear, and cytoplasmic proteins) in lung tissue were extracted and used for Western blot analysis. (A) Representative Western blot gel using an anti-NF-κB p65 polyclonal antibody. Equal sample loading was confirmed using a β-actin antibody. (B) Densitometric determination of NF-κB p65 levels in the total protein normalized to the β-actin level. Each bar represents mean ± SD (*n* = 6 for all groups; **p* < .05 vs. control group, ***p* < .05 vs. asthma group). (C) Proportions of NF-κB p65 protein in the nucleus and cytosol by densitometric analysis. Each bar represents mean ± SD (*n* = 6 for all groups; **p* < .05 vs. control group, ***p* < .05 vs. asthma group).

why vitamin D deficiency has been shown to be associated with accelerated declines in lung function (27–29).

The mechanism by which 1,25-(OH)₂D₃ mediated asthmatic airway remodeling was not fully elucidated. It has been shown that the VDR could interfere with the NF-κB p65 subunit and reduce the levels of IκBα protein, which directly results in its regulation of NF-κB activation (30–33). 1,25-(OH)₂D₃ binding to the VDR has been shown to regulate NF-κB activation and also modulate multiple biological activities both *in vivo* and *in vitro* (34–39). Our results extended these activities to lung tissues and showed that accompanied by the attenuated structural changes, 1,25-(OH)₂D₃ correspondingly inhibited NF-κB p65 nuclear translocation in the lung tissues of OVA-challenged mice.

Additionally, as a result of its inducing increased IκBα mRNA levels and a reduction of IκBα phosphorylation,

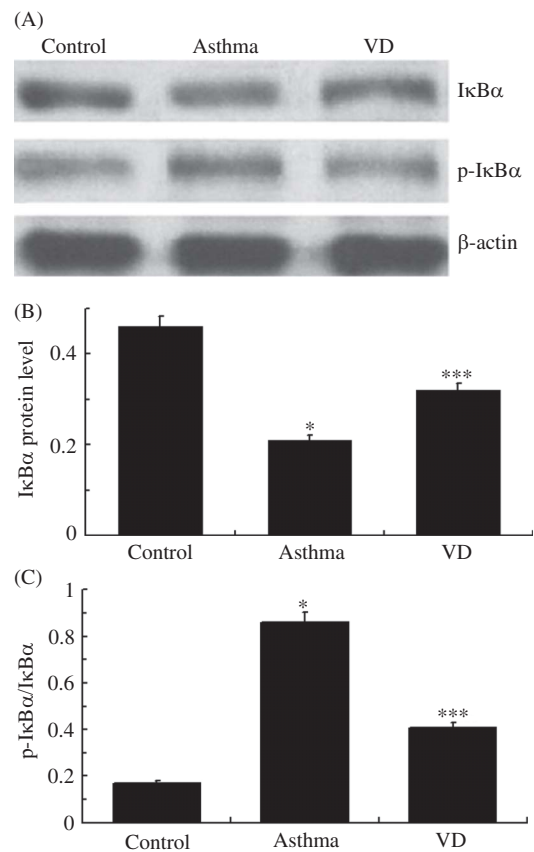


FIGURE 5.—1,25-(OH)₂D₃ increases the IκBα protein level but decreases its phosphorylation in a murine model of chronic asthma. The total protein was extracted from lung tissue and used for Western blot analysis. (A) Representative Western blot using an anti-IκBα or anti-phospho-IκBα (anti-p-IκBα) polyclonal antibody. Equal sample loading was confirmed using a β-actin antibody. (B) Densitometric determination of IκBα level in the total protein normalized to the β-actin level. Each bar represents mean ± SD (*n* = 6 for all groups; **p* < .05 vs. control group, ***p* < .05 vs. asthma group). (C) Densitometric determination of p-IκBα level in the total protein. Its level was calculated from the ratio of p-IκBα/total IκBα to remove the effect of varying amounts of total IκBα. Each bar represents mean ± SD (*n* = 6 for all groups; **p* < .05 vs. control group, ***p* < .05 vs. asthma group).

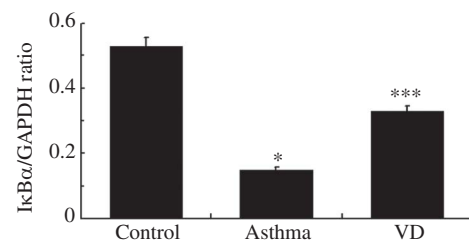


FIGURE 6.—1,25-(OH)₂D₃ decreases the IκBα mRNA level in a murine model of chronic asthma. Total RNA was extracted from lung tissue and used for real-time quantitative RT-PCR using a specific primer for IκBα. The IκBα mRNA expression level was normalized to the GAPDH mRNA level. Each bar represents mean ± SD (*n* = 6 for all groups; **p* < .05 vs. control group, ***p* < .05 vs. asthma group).

1,25-(OH)₂D₃ triggered an increase in pulmonary IκBα protein levels. This could be responsible for NF-κB p65 retention in the cytoplasm; that is, inhibition of NF-κB activation. Because NF-κB activation is supposedly a key event in asthmatic airway remodeling (9), the inhibition of

NF- κ B activation by 1,25-(OH)₂D₃ was probably an underlying mechanism for its protective effect on airway remodeling.

Of course, NF- κ B is a key transcription factor that also regulates immune and inflammatory responses in asthma (9). Thus, the inhibition of NF- κ B activation by 1,25-(OH)₂D₃ in OVA-challenged lung tissue might also establish a basis for its use for inflammation and immune regulation in asthma.

Recently, 1,25-(OH)₂D₃ was shown to possibly increase the therapeutic response to corticosteroids in corticosteroid-resistant asthma patients (40). Interestingly, persistent, excessive NF- κ B activation might hinder the clinical efficacy of corticosteroids (41,42). Therefore, apart from its ability to induce antigen-specific IL-10-secreting regulatory T cells (42), this study provides another explanation that 1,25-(OH)₂D₃ could target NF- κ B activation.

In summary, the results of this study demonstrated for the first time that 1,25-(OH)₂D₃ could directly reduce structural changes in the airways in a murine model of chronic asthma. This provides support for its use as a novel therapy to attenuate established airway remodeling in chronic asthma. Furthermore, our results suggested a possible role for 1,25-(OH)₂D₃ in regulating NF- κ B. However, more studies are needed for a better understanding of its mechanism of action.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the contents and writing of this article.

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