Article

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Vitamin D Attenuates Airway Inflammation via the Sema7A/Integrin-β1/NF-κB Axis in Asthma

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Background: Glucocorticoid resistance in asthma is frequently associated with persistent airway inflammation and oxidative stress. Although vitamin D has been reported to exert anti-inflammatory effects and restore steroid sensitivity, the underlying signaling mechanisms remain unclear. Given that Semaphorin 7A (Sema7A) and its receptor Integrin-β1 are implicated in immune activation and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)-mediated inflammatory responses, this study aimed to elucidate how Vitamin D confers protection against bronchial inflammation and redox imbalance, emphasizing the potential regulatory role of the Sema7A/Integrin-β1/NF-κB axis.

Methods: To model allergic airway disease, we utilized a murine asthma model induced by ovalbumin (OVA) and Lipopolysaccharide (LPS)-treated human bronchial epithelial (BEAS-2B) cells. Airway inflammation and oxidative stress were evaluated using enzyme-linked immunosorbent assay (ELISA), histopathological examination, and biochemical assays. *In vitro* cell proliferation, cytokine secretion, and reactive oxygen species (ROS) generation were measured. The expression of Sema7A, Integrin-β1, and NF-κB-related proteins was analyzed by Western blotting and reverse transcription quantitative PCR (RT-qPCR). Functional rescue assays were conducted by overexpressing Sema7A in BEAS-2B cells.

Results: In the OVA-induced asthma mouse model, vitamin D treatment markedly reduced Th2 cytokines (IL-4, IL-5, IL-13; all $p < 0.01$) while restoring interferon-gamma (IFN-γ) levels ($p < 0.05$), accompanied by attenuated airway inflammation and oxidative stress, as evidenced by elevated superoxide dismutase (SOD) and catalase (CAT) activities ($p < 0.05$) and decreased malondialdehyde (MDA) content ($p < 0.001$). In LPS-stimulated BEAS-2B cells, vitamin D at 1 nM and 10 nM similarly suppressed tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β) production, mitigated intracellular ROS accumulation, and rescued proliferative impairment (all $p < 0.05$). Mechanistically, Vitamin D inhibited activation of the Sema7A/Integrin-β1/NF-κB signaling cascade in both *in vivo* and *in vitro* systems, whereas Sema7A overexpression reactivated this pathway and partially diminished the anti-inflammatory effects of vitamin D.

Conclusion: Vitamin D mitigates airway inflammation and oxidative stress in allergic asthma by suppressing the Sema7A/Integrin-β1/NF-κB signaling pathway. These findings reveal a novel mechanistic insight into the anti-inflammatory action of vitamin D and highlight its therapeutic potential in allergic airway diseases.

Keywords: vitamin D; asthma; Sema7A; NF-κB pathway; oxidative stress; airway inflammation

Introduction

Asthma is a chronic respiratory disorder characterized by persistent inflammation, variable airflow limitation, airway hyperresponsiveness, and structural remodeling of the bronchial airways [1]. A growing body of evidence highlights the pivotal role of airway epithelial cells, not merely as a passive barrier but as active participants in immune modulation, through the secretion of cytokines, chemokines, and oxidative stress mediators such as reactive oxygen species (ROS) [2,3]. Notably, epithelial injury and subsequent dysfunction contribute significantly to asthma pathogenesis by amplifying inflammation and impairing tissue repair processes [4]. Lipopolysaccharide (LPS), a principal component of endotoxins derived from gram-negative bacteria, is commonly used to simulate inflammatory injury in airway epithelial cells and has been shown to worsen hyperresponsiveness in asthma [5].

Vitamin D, a lipid-soluble secosteroid hormone, has emerged as a potent immunomodulatory and antioxidant molecule [6,7]. Population-based studies have shown that low serum levels of vitamin D are associated with more severe asthmatic symptoms, compromised lung function, and increased airway inflammation [8]. Mechanistically, vitamin D is known to restore the Th1/Th2 immune equilibrium [9], inhibit nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)-mediated inflammatory signaling [10], and enhance antioxidant defenses [11]. However, the

precise molecular mechanisms through which vitamin D mitigates airway epithelial injury and inflammation, particularly within the framework of epithelial–immune interactions, remain incompletely understood.

Semaphorin 7A (Sema7A), a glycosylphosphatidylinositol-anchored protein, has been identified as a key regulator of immune cell recruitment and tissue inflammation [12]. Through interaction with Integrin-β1, Sema7A activates NF-κB signaling cascades, leading to the upregulation of proinflammatory cytokines [13]. While the Sema7A/Integrin-β1 axis has been implicated in various inflammatory disorders [14], its role in regulating epithelial functions in asthma pathophysiology remains largely unexplored.

In this study, we aimed to investigate the protective effects of vitamin D on airway inflammation and epithelial injury using an ovalbumin (OVA)-induced murine asthma model and LPS-stimulated human bronchial epithelial (BEAS-2B) cells. We further examined whether the Sema7A/Integrin-β1 axis mediates the immunomodulatory and antioxidant effects of vitamin D. By integrating *in vivo* and *in vitro* approaches, this study provides novel mechanistic insights into how vitamin D modulates airway inflammation and epithelial repair, potentially offering new therapeutic targets for asthma management.

Materials and Methods

Animal Model of Allergic Asthma and Vitamin D Treatment

A murine model of allergic asthma was established as previously described, with minor modifications [15]. Briefly, female BALB/c mice (6–8 weeks old; purchased from SPF Biotechnology, Beijing, China) were intraperitoneally sensitized with 0.5 mL of an emulsion containing 10 µg ovalbumin (OVA; S7951, Sigma-Aldrich, MO, USA) and 1 mg $KAl(SO_4)_2$ (A500755, Sangon Biotech, Shanghai, China) on Days 0 and 7. From Days 14 to 20, mice were challenged daily with aerosolized 1% OVA for 30 min. All animals were housed under specific pathogen-free (SPF) conditions at a controlled temperature (22 $\pm$ 2 °C) and relative humidity (50–60%). Mice were euthanized by intraperitoneal injection of sodium pentobarbital (150 mg/kg), followed by cervical dislocation to ensure death.

Mice were randomly assigned to four groups (n = 6 per group): Control (Con), OVA, Vitamin D low-dose (Vitamin D-L), and Vitamin D high-dose (Vitamin D-H). Mice in the Vitamin D-L group received daily intraperitoneal injections of 50 ng vitamin D ($1,25(OH)_2D_3$; D1530 Sigma-Aldrich, MO, USA) dissolved in ethanol and diluted in 300 µL phosphate-buffered saline (PBS) to achieve a final ethanol concentration of 0.9%. Mice in the Vitamin D-H group received 100 ng of vitamin D, following the same dilution protocol [16]. Both Vitamin D-L and Vitamin D-H groups received vitamin D administered 1 h before each OVA challenge from Days 14 to 20. The Con and OVA groups received an equivalent volume (300 µL) of PBS containing 0.9% ethanol as the vehicle control. All animals were sacrificed 24 h after the final OVA challenge. Bronchoalveolar lavage fluid (BALF), lung tissues, and serum were collected for subsequent analysis.

BALF and Cytokine Analysis

Following euthanasia, lungs were lavaged with PBS, and the recovered BALF was centrifuged to remove cellular debris. Cytokine levels in the BALF supernatant, including IL-4 (MU30385), IL-5 (MU30011), IL-13 (MU30012), and interferon-gamma (IFN-γ) (MU30338), were quantified using commercial ELISA kits (Bioswamp, Wuhan, China) following the manufacturer's instructions.

Histological Analysis

Lung tissues were excised, fixed in 4% paraformaldehyde at room temperature for 24 h, embedded in paraffin, and sectioned at a thickness of 5 µm. Hematoxylin and eosin (H&E) staining was performed to evaluate peribronchial inflammation and airway wall thickening. Images were captured under a light microscope (BX51, Olympus, Tokyo, Japan). For semi-quantitative analysis, a 5-point histological scoring system was employed to evaluate lung injury [17], extent of inflammation, and infiltration of inflammatory cells, defined as follows: 0 = normal, 1 = very mild, 2 = mild, 3 = moderate, 4 = marked, 5 = severe inflammation. Scoring was performed blindly by two independent pathologists with expertise in pulmonary pathology. For each lung section, a minimum of three randomly selected fields were examined under 100$\times$ magnification, and the mean score was calculated for each animal.

Oxidative Stress Assays

Whole blood was centrifuged at 1200 rpm for 15 min, and the obtained serum was aliquoted and stored at –80 °C until use. Levels of oxidative stress biomarkers, including malondialdehyde (MDA, S0131M), superoxide dismutase (SOD, S0101S), and catalase (CAT, S0051), were determined using commercial assay kits (Beyotime, Shanghai, China) following the manufacturer's protocols.

Cell Culture and Treatments

Human bronchial epithelial (BEAS-2B) cells were obtained from the American Type Culture Collection (CRL-3588, ATCC, VA, USA) and cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (11875093, Gibco, Shanghai, China) supplemented with 10% fetal bovine serum (FBS; A5670701, Gibco, Shanghai, China). Cells were maintained at 37 °C in a humidified incubator containing 5% CO_2. To ensure cell line authenticity and stability, BEAS-2B cells were authenticated by short tandem repeat (STR) profiling, and mycoplasma contamination was routinely screened using a PCR-based assay (EZ-PCR My-

coplasma Test Kit, 20-700-20, Biological Industries, Kibbutz Beit Haemek, Israel) before use in experiments. At 85–90% confluency, BEAS-2B cells were pretreated with vitamin D (1 or 10 nM) for 2 h, followed by stimulation with LPS (1 µg/mL; L658714, Aladdin, Shanghai, China) for the indicated durations. Experimental groups were as follows: (1) Control (Con) group: cells treated with vehicle (ethanol diluted in PBS); (2) LPS group: cells stimulated with LPS; (3) LPS + Vitamin D-L group: cells pretreated with 1 nM vitamin D for 2 h followed by LPS stimulation; and (4) LPS + Vitamin D-H group: cells pretreated with 10 nM vitamin D for 2 h followed by LPS stimulation.

Cell Viability Assay

Cell viability was determined using the Cell Counting Kit-8 (CCK-8; CK04, Dojindo, Kumamoto, Japan). BEAS-2B cells were seeded at 5×10^3 cells/well in 96-well plates and allowed to adhere overnight under standard culture conditions. Cells were then exposed to varying concentrations of vitamin D (0, 0.1, 1, 10, or 100 nM) for 24 h. Subsequently, 10% CCK-8 reagent was added to each well and incubated for an additional 2 h. Absorbance was measured at 450 nm using a microplate reader (Synergy HTX, BioTek, VT, USA) to determine cell viability.

ELISA for Inflammatory Cytokines

After treatment, culture supernatants from BEAS-2B cells were collected and centrifuged at 3000 rpm for 10 minutes at 4 °C to remove cell debris. The concentrations of tumor necrosis factor-α (TNF-α; YFXEH00034), interleukin-6 (IL-6; YFXEH00258), and interleukin-1β (IL-1β; YFXEH00259) in the supernatants were determined using ELISA kits (YiFeiXue, Nanjing, China) according to the manufacturer's instructions.

ROS Detection

Intracellular ROS levels were detected using the fluorescent probe 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA; C2938, Thermo Fisher Scientific, MA, USA). Following the indicated treatments, BEAS-2B cells were incubated with 10 µM DCFH-DA in serum-free medium for 30 min at 37 °C in the dark. After incubation, cells were washed three times with PBS to remove excess probe. The nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) for 5 min at room temperature. Fluorescence signals were visualized using a fluorescence microscope (BX51, Olympus, Tokyo, Japan).

EdU-Based Cell Proliferation Analysis

Cell proliferation was evaluated using the EdU incorporation assay (C0071S, Beyotime, Shanghai, China). Briefly, BEAS-2B cells were seeded in 12-well plates at a density of 1×10^5 cells per well and incubated overnight to allow for adherence. After the indicated treatments, cells were incubated with 10 µM EdU reagent for 2 h at 37 °C to label newly synthesized DNA. Subsequently, cells were fixed with 4% paraformaldehyde, permeabilized with 0.3% Triton X-100, and subjected to the click chemistry reaction according to the manufacturer's instructions. The nuclei were stained with DAPI for 5 min, and fluorescence images were acquired using a fluorescence microscope (BX51, Olympus, Tokyo, Japan), and EdU-positive cells were quantified.

Western Blot Analysis

Proteins were extracted from lung tissues or BEAS-2B cells using radioimmunoprecipitation assay (RIPA) lysis buffer. Equal amounts of total protein were separated by SDS-PAGE and subsequently transferred onto PVDF membranes. After blocking with 5% bovine serum albumin (BSA), the membranes were incubated overnight at 4 °C with the following primary antibodies: rat anti-Sema7A (1:500, MAB2068, R&D Systems, MN, USA), rabbit anti-Integrin-β1 (1:2000, ab179471, Abcam, Cambridge, UK), rabbit anti-p-NF-κB p65 (1:2000, 82335, Proteintech, Wuhan, China), rabbit anti-NF-κB p65 (1:5000, 80979, Proteintech, Wuhan, China), rabbit anti-p-IκBα (1:1000, 82349, Proteintech, Wuhan, China), rabbit anti-IκBα (1:5000, 10268, Proteintech, Wuhan, China), and mouse anti-β-actin (1:20,000, 66009, Proteintech, Wuhan, China). After incubation with horseradish peroxidase (HRP)-conjugated secondary antibodies (1:2000, SA00001-1, SA00001-2, SA00001-15, Proteintech, Wuhan, China), protein bands were visualized using enhanced chemiluminescence (ECL) reagent and quantified with ImageJ software (National Institutes of Health, MD, USA).

Sema7A Overexpression

To elucidate the functional role of Sema7A, the full-length SEMA7A coding sequence (NM_003612.5) was amplified by PCR and subcloned into the pcDNA3.1(+) expression vector (V79020, Invitrogen, CA, USA). BEAS-2B cells were transfected with either the SEMA7A overexpression plasmid or the empty vector control using Lipofectamine 3000 (L3000001, Invitrogen, CA, USA) following the manufacturer's instructions. Forty-eight hours post-transfection, cells were treated with vitamin D and LPS. The efficiency of SEMA7A overexpression was confirmed by reverse transcription quantitative PCR (RT-qPCR) and western blot analysis.

RT-qPCR

Total RNA was isolated from BEAS-2B cells using TRIzol reagent (15596026CN, Invitrogen, CA, USA) following the manufacturer's instructions. First-strand cDNA was synthesized from 2 µg of total RNA using a reverse transcription kit (4374966, Invitrogen, CA, USA). RT-qPCR was performed with SYBR Green PCR Master Mix (4367659, Thermo Fisher Scientific, MA, USA). GAPDH served as the internal control, and relative gene expres-

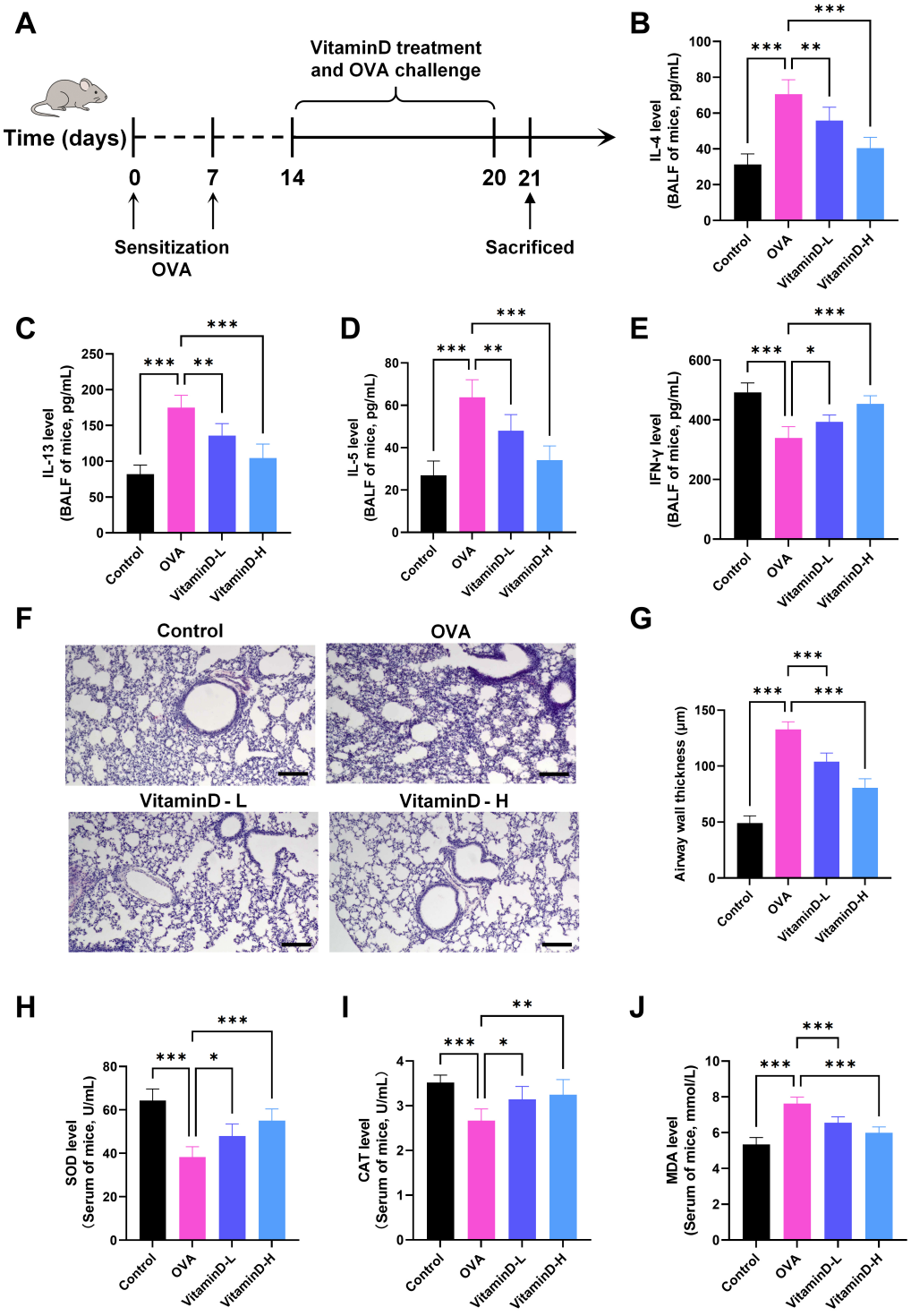

Fig. 1. Vitamin D mitigates pulmonary inflammation and oxidative imbalance in a murine model of asthma. (A) Schematic illustration of the experimental protocol used to induce ovalbumin (OVA)-mediated allergic airway inflammation in mice. Levels of (B) IL-4, (C) IL-13, and (D) IL-5 in bronchoalveolar lavage fluid (BALF) measured by enzyme-linked immunosorbent assay (ELISA). (E) Interferon-gamma (IFN-γ) levels in BALF. (F) Representative hematoxylin and eosin (H&E)-stained lung tissue sections showing inflammatory changes (scale bar: 100 μm). (G) Measurement of airway wall thickness in mice. Serum oxidative stress markers: (H) superoxide dismutase (SOD), (I) catalase (CAT), and (J) malondialdehyde (MDA). Data are presented as mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

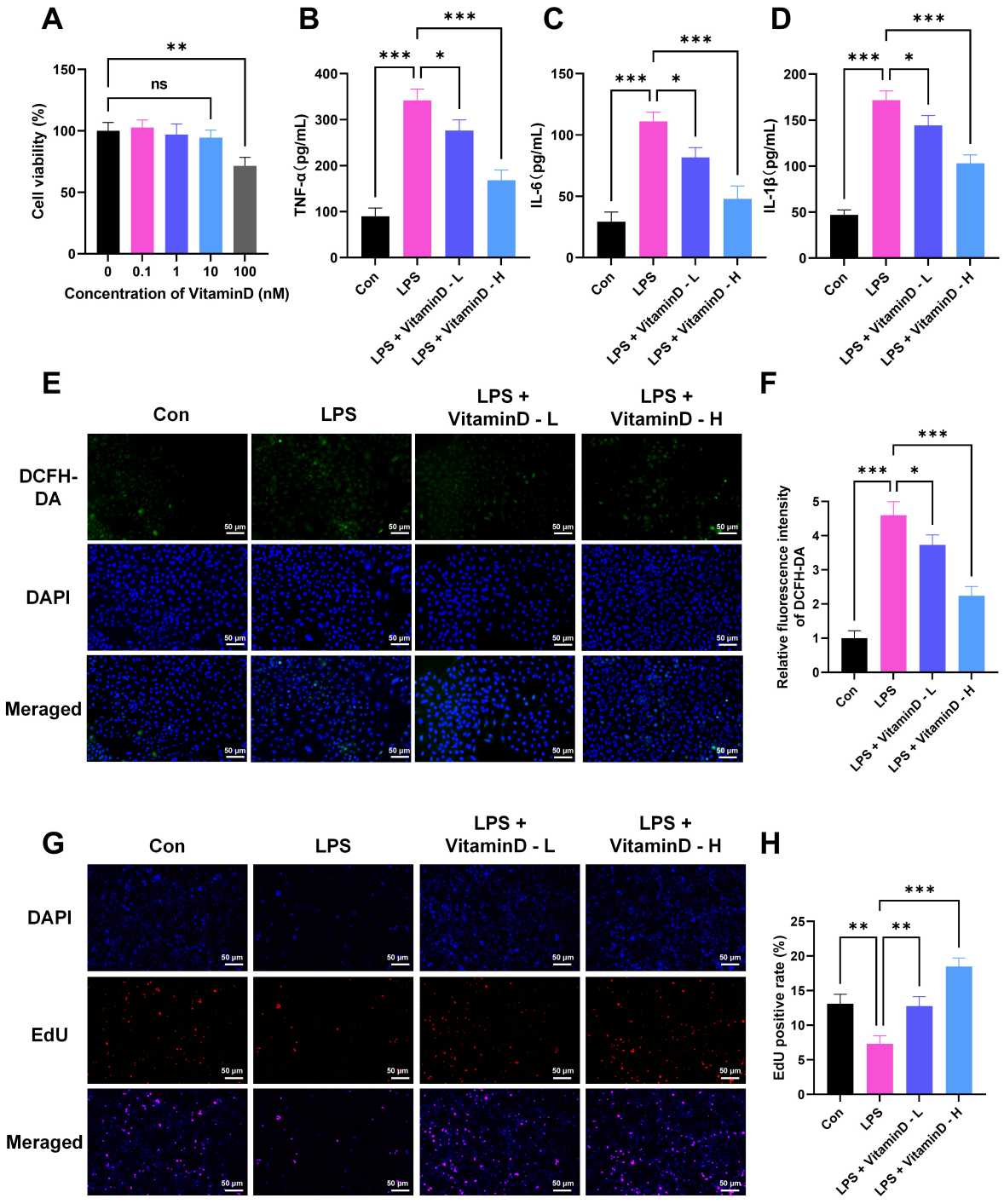

Fig. 2. Vitamin D reduces inflammatory cytokine production, oxidative stress, and promotes proliferation in BEAS-2B cells. (A) Cell viability of BEAS-2B cells treated with increasing concentrations of vitamin D, assessed using the CCK-8 assay. ELISA quantification of (B) TNF-α, (C) IL-6, and (D) IL-1β levels in cell culture supernatants. (E) Representative fluorescence images of intracellular ROS levels (Scale bar: 50 μm). (F) Quantification of ROS fluorescence intensity. (G) Representative EdU staining images showing proliferating cells (scale bar: 50 μm). (H) Quantitative analysis of EdU-positive cell percentages. ns $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. BEAS-2B, human bronchial epithelial; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; IL-1β, interleukin-1β; ROS, reactive oxygen species.

sion levels were calculated using the $2^{-\Delta\Delta Ct}$ method. The primer sequences used were as follows: *Sema7A*, forward 5′-CTTCTTCCGAGAGGACAATCCTG-3′, reverse 5′-GTGTTCCACTTGGAGACTGACAG-3′; *GAPDH*, forward 5′-GTCTCCTCTGACTTCAACAGCG-3′, reverse 5′-ACCACCCTGTTGCTGTAGCCAA-3′.

Statistical Analysis

All experiments were performed in triplicate or independently repeated at least three times. Data are presented as mean ± standard deviation (SD). Statistical analyses were conducted using GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA). For multiple group comparisons, one-way analysis of variance (ANOVA) was employed after confirming homogeneity of variances with the Brown-Forsythe test. When the assumptions of normality or variance homogeneity were violated, the non-parametric Kruskal-Wallis test, followed by Dunn's multiple comparisons test, was applied. A p-value < 0.05 was considered statistically significant.

Results

Vitamin D Alleviates Airway Inflammation and Oxidative Stress in Asthmatic Mice

To examine the protective role of vitamin D against allergic asthma, a murine model was established through OVA sensitization followed by antigenic challenge (Fig. 1A). ELISA analysis revealed significantly elevated levels of IL-4, IL-13, and IL-5 in the BALF of OVA-induced mice ($p < 0.001$), whereas vitamin D treatment markedly reduced these cytokines ($p < 0.01$) (Fig. 1B–D). Conversely, the level of IFN-γ, which was markedly suppressed in asthmatic mice ($p < 0.001$), was significantly restored following vitamin D treatment ($p < 0.05$) (Fig. 1E). H&E staining further revealed that vitamin D administration mitigated airway inflammatory infiltration and reduced airway wall thickness ($p < 0.001$) (Fig. 1F,G). Moreover, serum oxidative stress markers indicated that vitamin D significantly increased SOD and CAT activities ($p < 0.05$) (Fig. 1H,I) while reducing MDA content ($p < 0.001$) (Fig. 1J). Collectively, these findings suggest that vitamin D attenuates both inflammatory cytokine production and oxidative imbalance in OVA-challenged mice.

Vitamin D Suppresses Inflammatory Cytokine Production, Oxidative Stress, and Proliferation Impairment in LPS-Treated BEAS-2B Cells

To further investigate the anti-inflammatory and antioxidant properties of vitamin D *in vitro*, BEAS-2B cells were exposed to a concentration gradient of vitamin D (0, 0.1, 1, 10, 100 nM). CCK-8 assay demonstrated that cell proliferation remained largely unaffected within the 0.1–10 nM range ($p > 0.05$), while a significant reduction in viability was observed at 100 nM ($p < 0.01$) (Fig. 2A). Based on these results, 1 nM and 10 nM vitamin D were selected for subsequent experiments.

ELISA results demonstrated that vitamin D significantly suppressed the secretion of TNF-α, IL-6, and IL-1β in the culture medium ($p < 0.05$) (Fig. 2B–D). Additionally, ROS fluorescence staining revealed that vitamin D markedly reduced intracellular ROS accumulation ($p < 0.05$) (Fig. 2E,F).

To evaluate the influence of vitamin D on epithelial cell proliferation, EdU incorporation assays were performed. LPS stimulation significantly reduced the proportion of EdU-positive cells, suggesting impaired proliferative capacity. In contrast, treatment with low (1 nM) and high (10 nM) concentrations of vitamin D markedly increased the number of EdU-positive cells ($p < 0.01$), suggesting that vitamin D effectively restores the proliferative potential of LPS-injured BEAS-2B cells (Fig. 2G,H). Collectively, these findings demonstrate that vitamin D inhibits inflammatory responses and oxidative stress while preserving epithelial cell viability and promoting regeneration under inflammatory conditions.

Vitamin D Modulates the Sema7A/Integrin-β1/NF-κB Signaling Pathway In Vivo and In Vitro

To elucidate the molecular mechanisms underlying the anti-inflammatory effects of vitamin D, we examined the expression of Sema7A, Integrin-β1, and key components of the NF-κB signaling pathway. Immunoblotting of lung tissues from asthmatic mice revealed elevated expression of Sema7A and Integrin-β1, accompanied by enhanced phosphorylation of NF-κB p65 and its upstream inhibitor IκBα ($p < 0.01$). These pathological alterations were significantly attenuated following vitamin D administration ($p < 0.01$) (Fig. 3A–E). Consistently, in LPS-treated BEAS-2B cells, vitamin D markedly reduced the expression of Sema7A, Integrin-β1, and phosphorylated NF-κB p65 and IκBα ($p < 0.01$) (Fig. 3F–J). Collectively, these findings indicate that vitamin D suppresses airway inflammation, at least in part, by negatively modulating the Sema7A/Integrin-β1/NF-κB signaling axis.

Sema7A Overexpression Activates the Integrin-β1/NF-κB Pathway and Attenuates the Anti-Inflammatory Effects of Vitamin D

To further confirm the role of Sema7A in vitamin D-mediated anti-inflammatory signaling, Sema7A was overexpressed in BEAS-2B cells. Western blot and RT-qPCR analyses confirmed successful upregulation of Sema7A at both the protein and mRNA levels ($p < 0.001$) (Fig. 4A–C). Functional rescue experiments showed that overexpression of Sema7A significantly increased the levels of Integrin-β1 and activated NF-κB pathway components, including p-NF-κB p65 and p-IκBα ($p < 0.001$) (Fig. 4D–

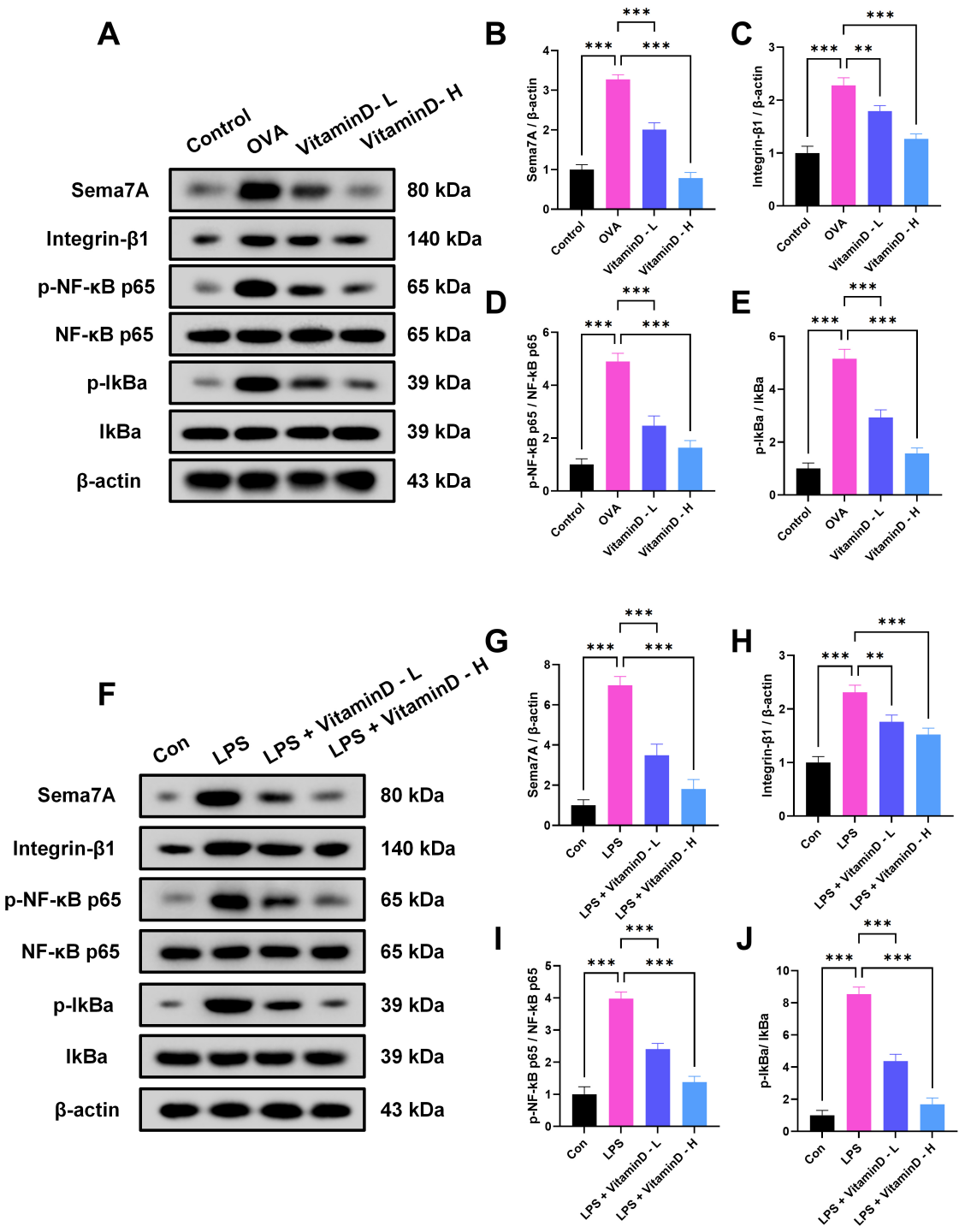

Fig. 3. Vitamin D modulates the Sema7A/Integrin-β1/NF-κB signaling pathway in asthmatic mice and LPS-treated BEAS-2B cells. (A) Western blot analysis of Sema7A, Integrin-β1, p-NF-κB p65, NF-κB p65, p-IκBα, and IκBα expression in lung tissues from experimental groups. Quantitative analysis of protein expression in mouse lung tissues: (B) Sema7A / β-actin, (C) Integrin-β1 / β-actin, (D) p-NF-κB p65/NF-κB p65, (E) p-IκBα/IκBα. (F) Western blot analysis of Sema7A, Integrin-β1, p-NF-κB p65, NF-κB p65, p-IκBα, and IκBα protein expression in BEAS-2B cells. Quantitative analysis of protein levels in BEAS-2B cells: (G) Sema7A/β-actin, (H) Integrin-β1/β-actin, (I) p-NF-κB p65/NF-κB p65, (J) p-IκBα/IκBα. $^{**}p < 0.01$, $^{***}p < 0.001$. Sema7A, Semaphorin 7A; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; LPS, Lipopolysaccharide.

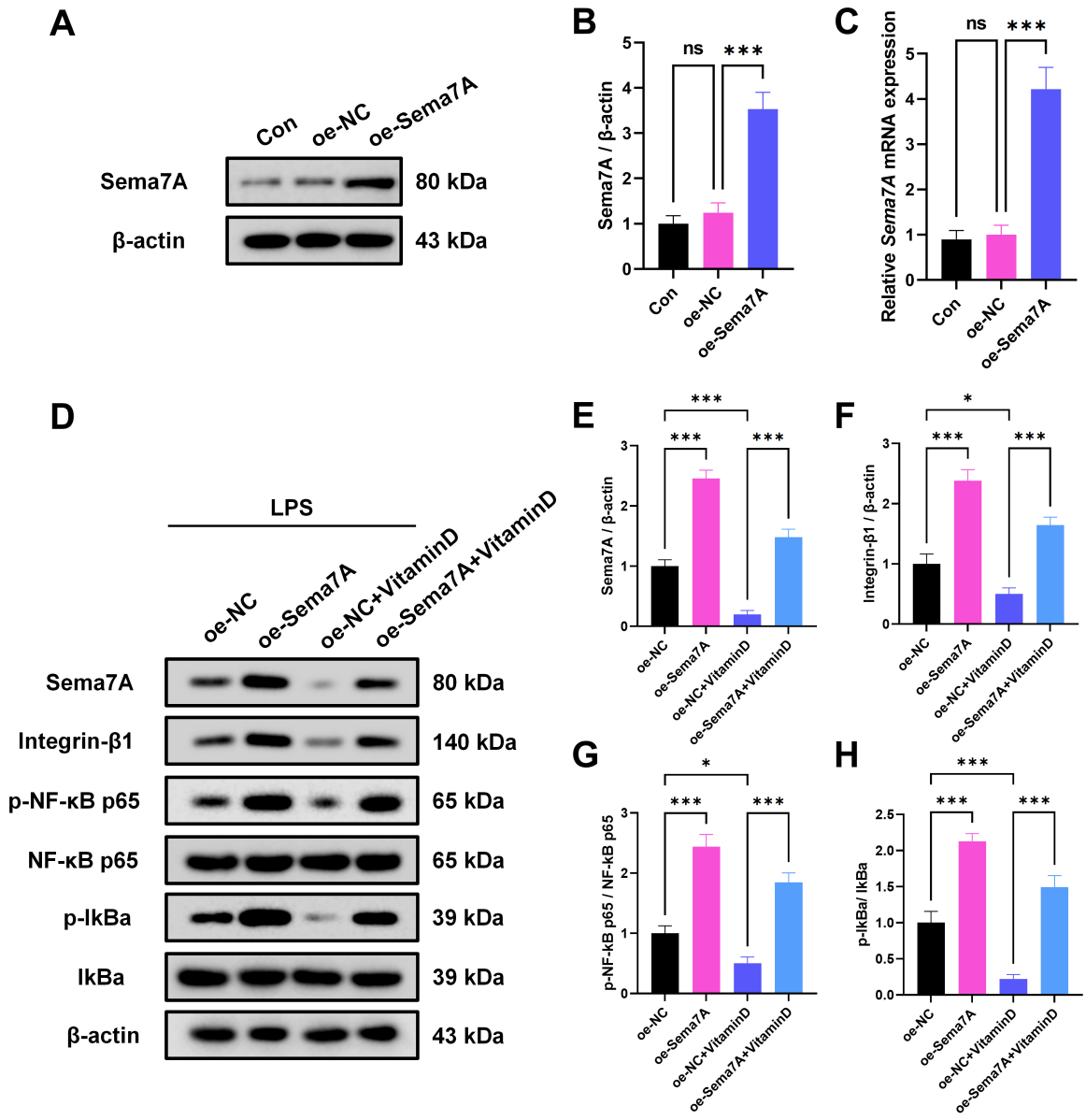

Fig. 4. Sema7A overexpression activates Integrin-β1/NF-κB signaling in airway epithelial cells. (A) Western blot detection of Sema7A protein following transfection with an overexpression plasmid. (B) Quantification of Sema7A protein levels. (C) Reverse transcription quantitative PCR (RT-qPCR) analysis of *Sema7A* mRNA expression. Rescue experiments: (D) Western blot analysis of Sema7A, Integrin-β1, p-NF-κB p65, NF-κB p65, p-IκBα, and IκBα expression in BEAS-2B cells under different treatment conditions. Quantitative analysis of protein expression: (E) Sema7A/β-actin, (F) Integrin-β1/β-actin, (G) p-NF-κB p65/NF-κB p65, (H) p-IκBα/IκBα. ns $p > 0.05$, *$p < 0.05$, ***$p < 0.001$.

H). Notably, co-treatment with vitamin D partially reversed these effects, indicating that Sema7A functions upstream of Integrin-β1/NF-κB and serves as a critical mediator of vitamin D-dependent signaling. These findings support that the Sema7A/Integrin-β1/NF-κB pathway is a functional target through which vitamin D exerts its anti-inflammatory and antioxidant effects.

Discussion

In this study, we identified a novel anti-inflammatory mechanism through which vitamin D attenuates allergic airway inflammation and oxidative stress by inhibiting the Sema7A/Integrin-β1/NF-κB signaling axis. Using a murine model of asthma and LPS-challenged airway epithelial cells, we demonstrated that vitamin D significantly reduced Th2 cytokine production, suppressed ROS genera-

tion, restored epithelial proliferation, and enhanced antioxidant capacity. Mechanistic analyses further revealed that overexpression of Sema7A reversed the protective effects of vitamin D, confirming the functional relevance of this signaling pathway. These findings not only advance the mechanistic understanding of the immunoregulatory role of vitamin D but also highlight its translational potential as an adjunctive therapeutic approach in asthma management, especially among patients exhibiting epithelial dysfunction and corticosteroid resistance.

Consistent with our hypothesis, *in vivo* administration of vitamin D significantly suppressed the OVA-induced elevation of Th2-associated cytokines (IL-4, IL-5, and IL-13) in BALF and restored IFN-γ expression, suggesting a shift toward a Th1-dominant immune profile. Histological analyses supported these findings, revealing reduced peribronchial inflammatory infiltration and decreased airway wall hypertrophy following vitamin D treatment. Concurrently, serum assays indicated a marked decline in oxidative stress, characterized by increased activities of antioxidant enzymes (SOD and CAT) and decreased MDA levels. These findings are consistent with previous reports highlighting the immunomodulatory and antioxidant properties of vitamin D in allergic airway diseases [18,19].

Given the pivotal role of epithelial injury and repair in asthma pathogenesis, we employed LPS-treated BEAS-2B cells as an *in vitro* model to replicate airway injury and inflammatory responses in the epithelium [20,21]. Our *in vitro* findings indicated that vitamin D at physiologically relevant concentrations (1–10 nM) maintained normal epithelial cell viability, indicating favorable biocompatibility. Notably, vitamin D treatment markedly attenuated LPS-induced proinflammatory responses, including TNF-α, IL-6, and IL-1β, while concurrently decreasing intracellular ROS accumulation. Moreover, vitamin D restored the proliferative potential of LPS-injured epithelial cells, evidenced by a notable increase in EdU-positive cells. Collectively, these results indicate that vitamin D mitigates inflammation and oxidative stress while promoting epithelial regeneration, thereby alleviating cellular dysfunction induced by proinflammatory stimuli, without inducing cytotoxic effects within its physiological concentration range.

Mechanistically, we identified the Sema7A/Integrin-β1/NF-κB signaling axis as a key pathway through which vitamin D exerts its biological effects. In both the lung tissues of asthmatic mice and LPS-treated BEAS-2B cells, vitamin D significantly downregulated Sema7A and Integrin-β1 expression, accompanied by decreased phosphorylation of NF-κB p65 and IκBα, thereby inhibiting the classical NF-κB signaling cascade. Given the established role of Sema7A in immune cell recruitment and the integrin-mediated activation of NF-κB signaling, our findings support a model in which vitamin D suppresses airway inflammation by disrupting this proinflammatory signaling cascade.

Furthermore, we observed that Sema7A overexpression in BEAS-2B cells elevated Integrin-β1 levels and enhanced NF-κB activation, which significantly attenuated the anti-inflammatory effects of vitamin D. These rescue experiments provide compelling evidence that Sema7A acts as a key upstream regulator mediating the proinflammatory responses antagonized by vitamin D. Our results align with previous studies demonstrating that vitamin D alleviates airway inflammation by suppressing NF-κB activity and modulating the Th1/Th2 cytokine balance [22,23]. For instance, earlier investigations have revealed that vitamin D enhances corticosteroid responsiveness and reduces eosinophilic inflammation via inhibition of NF-κB signaling pathways [24]. However, our study uniquely identifies the Sema7A/Integrin-β1 axis as a previously underexplored molecular interface linking vitamin D to NF-κB signaling. Whereas previous studies have primarily focused on the immunosuppressive effects of vitamin D on immune cells such as T cells and dendritic cells [25–27], our findings highlight epithelial cells as critical effectors of vitamin D signaling in airway injury. By integrating epithelial cell biology with immune regulation, our study expands the mechanistic understanding of asthma pathophysiology and positions vitamin D as a potential modulator of epithelial resilience.

Building on these findings, we hypothesize that vitamin D may directly repress Sema7A transcription through its canonical genomic pathway. Upon binding to vitamin D, the vitamin D receptor (VDR) forms a heterodimer with the retinoid X receptor (RXR) and regulates gene transcription by interacting with vitamin D response elements (VDREs) within target gene promoters. Although chromatin-level interactions were not evaluated in the present study, future investigations should aim to determine whether VDRE-like motifs exist within the Sema7A promoter and whether VDR directly binds these regions. Experimental approaches such as *in silico* promoter analysis, chromatin immunoprecipitation (ChIP), and luciferase reporter assays will be critical to validate this potential direct regulatory mechanism. Confirming this hypothesis would establish a more complete causal framework linking vitamin D–VDR engagement to Sema7A suppression, ultimately suppressing Integrin-β1/NF-κB signaling and enhancing epithelial anti-inflammatory responses.

From a translational perspective, these findings may hold significant relevance for the clinical management of asthma, particularly during acute exacerbations where rapid-onset inflammation is a defining feature. Although vitamin D is typically administered orally as a systemic immunomodulator, our results suggest that localized pulmonary delivery may confer more immediate and targeted anti-inflammatory benefits. However, the development of inhalable vitamin D formulations remains technically challenging due to their lipophilic nature, poor aqueous solubility, and susceptibility to degradation. To address these limitations, innovative delivery platforms, such as lipid-

based nanoparticles, dry powder inhalers, or micelle-based aerosols, may be required to achieve efficient pulmonary deposition and bioavailability. Despite these formulation barriers, our findings provide preclinical justification for further investigation into inhaled vitamin D as a therapeutic adjunct in asthma, potentially enabling faster symptom control with fewer systemic side effects.

Beyond its independent anti-inflammatory properties, our findings suggest that vitamin D may act synergistically with glucocorticoids to overcome inflammation-induced steroid resistance. Given that NF-κB overactivation is known to impair glucocorticoid responsiveness in inflammatory settings, it is plausible that persistent activation of the Sema7A/NF-κB axis similarly disrupts glucocorticoid receptor (GR) signaling, for example, by promoting GR phosphorylation or diverting essential transcriptional coactivators. By suppressing Sema7A-mediated NF-κB activation, vitamin D may restore GR transactivation capacity, lower the threshold for glucocorticoid responsiveness, and ultimately enhance clinical efficacy. Future investigations evaluating combined vitamin D-glucocorticoid therapy in steroid-resistant asthma models will be essential to validate this hypothesis.

Despite these promising implications, several limitations of this study should be acknowledged. First, although the murine model reproduces key features of allergic asthma, it may not fully capture the complexity of human disease. Second, while our *in vitro* experiments support the role of Sema7A in epithelial inflammation, further validation using human primary airway cells or clinical samples is warranted. Lastly, additional signaling pathways, including nuclear factor erythroid 2–related factor 2 (Nrf2) or mitogen-activated protein kinase (MAPK), may also interface with vitamin D signaling and warrant further investigation to comprehensively elucidate the network of regulatory mechanisms involved.

Conclusion

This study provides new insights into how vitamin D confers protection against allergic airway injury, identifying the Sema7A/Integrin-β1/NF-κB signaling cascade as a pivotal pathway underlying its immunoregulatory and oxidative stress-mitigating functions. Our findings expand the mechanistic understanding of the protective functions of vitamin D and suggest that modulation of this signaling axis represents a promising therapeutic strategy for asthma and related respiratory inflammatory disorders.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Author Contributions

ZP, HL and ZW designed the research study. ZP, XL, AZ, and XX performed the experiments. ZP, HL, and ZW collected and analyzed the data. XL, AZ, XX and HL drafted the manuscript, ZP and ZW critically revised it for important intellectual content. All authors gave final approval of the version to be published. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity are appropriately investigated and resolved.

Ethics Approval and Consent to Participate

All animal procedures were approved by the Animal Ethics Committee of South Zhejiang Institute of Radiation Medicine and Nuclear Technology Applications (Approval No. ZFY20250111) and were conducted in compliance with institutional guidelines.

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Conflict of Interest

The authors declare no conflict of interest.

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