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Lyn Mitigates Mouse Airway Remodeling by Downregulating the TGF- β 3 Isoform in House Dust Mite Models

Guoping Li,^{*,†,‡} John Fox, III,^{*} Zhigang Liu,[‡] Jun Liu,[§] George F. Gao,[§] Yang Jin,[¶] Hongwei Gao,^{||} and Min Wu^{*,#}

Chronic airway remodeling is a serious consequence of asthma, which is caused by complex but largely unknown mechanisms. Despite versatile functions, the role of Lyn in chronic airway remodeling remains undefined. Using Lyn^{-/-} mice, we show that continual exposure (for 8 wk) of house dust mite extracts induced a severe phenotype of chronic airway remodeling, including exacerbated mucus production, collagen deposition, dysregulated cytokine secretion, and elevated inflammation. Strikingly, a significant increase in TGF- β 3 rather than TGF- β 1 was observed in Lyn^{-/-} mouse lungs compared with lungs in wild-type mice. Furthermore, TGF- β 3 neutralizing Abs not only inhibited the expression of STAT6 and Smad2/3 but also decreased phosphorylation of Smad2 and NF- κ B in Lyn^{-/-} mouse lungs. In addition, both recombinant and adenoviral TGF- β 3 significantly promoted epithelial-to-mesenchymal transition and intensified collagen I production and MUC5AC expression. Further examination of chronic asthma patients showed that a decreased Lyn correlated with the severity of airway inflammation and mucus hypersecretion. Finally, Lyn may critically regulate airway remodeling by directly interacting with TGF- β 3. Collectively, these findings revealed that Lyn regulates TGF- β 3 isoform and modulates the development of airway remodeling, which may have therapeutic implications for severe chronic asthma. *The Journal of Immunology*, 2013, 191: 000–000.

Airway remodeling is a key feature of severe asthma, which affects 300 million people worldwide (1) and is associated with significant morbidity and health costs (2). Airway remodeling is characterized as epithelial detachment, subepithelial fibrosis, increased smooth muscle mass, goblet cell hyperplasia, and angiogenesis (3). Further, airway remodeling is

currently incurable and presents a serious problem in asthma management. Interactions between the immune system and lung structural cells are critical for initiation and development of airway inflammation and remodeling (4). The airway epithelium secretes multiple cytokines, chemokines, and growth factors during exposure to inhaled allergens. In addition, epithelial-to-mesenchymal transition (EMT) is a process whereby epithelial cells lose their markers while gaining mesenchymal symbolic molecules, which may contribute to pathophysiological alterations during airway remodeling. Chronic respiratory aeroallergen exposure in mice induces EMT in the large airways (5). Airway epithelium is critically involved in airway remodeling through the process of EMT following environmental toxicant or allergen challenge. Lasting and dynamic airway epithelial alterations may contribute to airway remodeling via EMT in asthma (6).

TGF- β is one of the major mediators involved in airway remodeling in asthma. Previous studies suggest that TGF- β could be a target for therapeutic intervention for airway remodeling in chronic asthma (5, 7). TGF- β isoforms in mammals include TGF- β 1, TGF- β 2, and TGF- β 3, which are expressed in bronchial epithelium, with TGF- β 1 and TGF- β 3 being secreted by macrophages. Bronchial epithelium, macrophages, and smooth muscle cells in normal mouse lungs can be strongly stained for TGF- β 3. Furthermore, other cells in the lungs may become TGF- β 3 positive under inflammatory conditions (8–11). Previous studies showed that airway tissue remodeling may involve TGF- β 2 or TGF- β 1, but not TGF- β 3, in epithelial cell culture (12, 13) as well as in asthmatic patients (14, 15). However, another report showed an increase in TGF- β 3 mRNA in allergic airway inflammation in mouse lungs, but no changes in mRNA for TGF- β 1 or TGF- β 2 (16). In an allergic model induced by house dust mite (HDM) extracts, TGF- β (primarily β 1) regulates airway inflammatory response instead of airway remodeling (17). Because a definite role for each of the TGF- β isoforms in asthmatic pathogenesis remains elusive, further studies are warranted. TGF- β family members mediate intracellular signaling via Smad proteins or MAPK, resulting in increased transcription of target genes (7, 16). Chronic exposure to aeroallergens induces

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Abbreviations used in this article: AM, alveolar macrophage; BAL, bronchoalveolar lavage; EMT, epithelial-to-mesenchymal transition; HDM, house dust mite; PAS, periodic acid-Schiff; RIPA, radioimmunoprecipitation assay; SH, Src homology; α -SMA, α -smooth muscle actin; WT, wild-type.

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EMT through the TGF- β 1 signaling pathway in the large airway of mice (5). However, little is known about the expression pattern and role of TGF- β 3 in airway remodeling.

Nonreceptor tyrosine kinases, such as Lyn, play a critical role in a variety of processes in response to receptor activation (18). We and others (19–22) have demonstrated that Lyn coordinates the activity of NF- κ B, PI3K, and Akt in host defense against bacterial infections. In asthma, Lyn peptide inhibitor blocks eosinophil differentiation, survival, and airway influx (23). In an OVA allergic model, Lyn^{-/-} mice exhibited asthma-like characteristics, which can be conferred by adoptive transfer of dendritic cells from Lyn^{-/-} mice (24). Aged Lyn^{-/-} mice manifested elevated serum IL-2 and diminished TGF- β 1 (25). An early examination indicates that TGF- β is involved in the phosphorylation of Lyn (26); and recent study indicates a link of Lyn to TGF- β in chronic myeloid leukemia (27). However, the role of Lyn in regulating TGF- β in chronic airway remodeling is unknown.

In this article, we examined the role of Lyn in airway remodeling in Lyn^{-/-} mice after HDM challenges and revealed that HDM-challenged Lyn^{-/-} mice exhibited aggressive airway remodeling phenotypes, including mucus hypersecretion, collagen deposition, α -smooth muscle actin (α -SMA) expression, and thickening of the peribronchial smooth muscle layer. Mechanistically, we found that Lyn deficiency resulted in a dramatic increase in TGF- β 3 and that neutralizing this protein significantly attenuated the features of airway remodeling. Furthermore, the TGF- β 3 pathway may promote EMT, which in turn upregulates collagen expression and mucus secretion in human epithelial cells.

Materials and Methods

Reagents

Nonsilencing siRNA control and Lyn siRNA were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The Abs for histology were purchased from Santa Cruz Biotechnology: fibronectin (clone no.: A-11; cat. no.: sc-271098), E-cadherin (clone no.: N-20; cat. no.: sc-1500), MUC5AC (mucin 5AC, clone no.: K-20; cat. no.: sc-16903), and collagen I (clone no.: COL-1; cat. no.: sc-59772) (Santa Cruz Biotechnology). The ELISA kits of TNF- α , IL-4, and IL-10 were purchased from R&D Systems (Minneapolis, MN). MUC5AC-pGL3 luciferase vector was constructed by our laboratory based on the pGL3 luciferase reporter vector (Promega) following the manufacturer's instructions.

Immunization and challenge protocol

Lyn^{-/-} mice and wild-type (WT) mice were sensitized by i.p. injection with 20 μ g HDM (cat. no.: B64; *Dermatophagoides farinae*, GREER Laboratories, Lenoir, NC) and 1 mg aluminum hydroxide on days 0 and 14. Mice were challenged with intranasal instillation of 10 μ g HDM in 30 μ l PBS buffer 3 times per week from week 3 to week 8 (for a duration of 5 wk). This type of HDM extract, as a natural allergen model, is widely used and evaluated by our laboratory (28). Lyn^{-/-} mice and WT mice in the control group were given PBS alone. Mice were sacrificed at 24 h following final intranasal challenge by i.p. injection of a ketamine mixture (40 mg/kg ketamine-HCl and 5 mg/kg xylazine-HCl). Lung tissues were kept in liquid nitrogen or fixed in 10% neutral-buffered formalin and embedded in paraffin. Lyn^{-/-} mice with C57BL/6J and J129/F genetic backgrounds were generated as previously described (19) and generously provided by Dr. S. Li of University of Massachusetts (Worcester, MA). Mice were backcrossed to a C57BL/6 background for seven generations and maintained under pathogen-free conditions, and experiments were initiated when mice were 6 to 8 wk of age. Genotyping was performed by PCR and Western blotting. Mice were housed in the University of North Dakota Center for Biomedical Research. All animal experiments were performed in accordance with the guidelines of the University of North Dakota Institutional Animal Care and Use Committee.

Ab administration

Neutralizing Abs for TGF- β 3 raised against an epitope of the C-terminal 345–375 aa (mouse monoclonal B-11, sc-166861), IL-4 (rat monoclonal, 11B11, sc-32242), normal mouse control IgG (sc-2025) were obtained

from Santa Cruz Biotechnology and administered i.p. according to a previous report with modifications at a dose of 20 μ g in 0.5 ml sterile PBS (28). Lyn^{-/-} mice were sensitized by i.p. injection with 20 μ g HDM and 1 mg aluminum hydroxide gel (cat. no.: A8222; Sigma-Aldrich) on days 0 and 14. Mice were challenged with intranasal instillation of 10 μ g HDM in 100 μ l PBS buffer every other day for 21 d. TGF- β 3, IL-4, or control Ab (depending on the group) was administered every other day beginning 24 h prior to the HDM exposure until the last HDM exposure. Mice were sacrificed 24 h following the last challenge. Lung tissues were kept at -80°C for Western blot or fixed in 10% neutral-buffered formalin and embedded in paraffin for the standard H&E stain.

Human lung tissue

The study included seven patients with asthma and seven healthy controls who were recruited in an ongoing study at the Affiliated Hospital of Luzhou Medical College (Sichuan, China) between July 2011 and January 2012. This study was approved by the Luzhou Medical College Ethics Committee. The diagnosis of asthma was based on the asthma guidelines given in *National Heart Lung and Blood Institute Guidelines for the Diagnosis and Management of Asthma* (Bethesda, MD, National Institutes of Health publication 97-4051). (Clinical characteristics are presented in Fig. 7.) Lung tissues were kept at -80°C before immunofluorescent staining.

ELISA for cytokines and allergen-specific IgE

The mouse trachea was cannulated and lavaged four times with 1.0 ml PBS each. Bronchoalveolar lavage (BAL) fluid was stored at -70°C . Cytokine levels were determined in triplicate samples of BAL fluid from each animal by ELISA, with a sensitivity limit in picogram ranges (19). Allergen-specific IgE was evaluated by ELISA, using our previously described methods (28).

Airway inflammation and airway remodeling evaluation

Cells in BAL fluid were enumerated with a hemocytometer. Samples were centrifuged ($500 \times g$ for 5 min at 4°C) and resuspended. Slides were air dried, and stained by HEMA 3 STAT PACK (Fisher Scientific Company, Pittsburgh, PA). Differential cell counts were performed in duplicate on coded slides for 200 cells from each sample. The lung tissues were fixed in 10% neutral-buffered formalin and embedded in paraffin. Sections (5 μ m) of specimens were stained with standard H&E methods to evaluate the tissue histological alterations, including inflammation, airway thickening, and angiogenesis. Lung sections were also stained with periodic acid-Schiff (PAS) reagent for detecting airway mucus production. Masson's trichrome staining was used for assessment of subepithelial fibrosis. The tissues were assessed for general morphology and cellular infiltration. Images were obtained using an 80i Nikon Eclipse Microscope (Melville, NY). The degree of cellular infiltration was scored using previously described methods (28, 29). The index was calculated by multiplying severity by extent, with a maximum possible score of 9. Masson's trichrome staining was used to detect peribronchial collagen deposition. A score ranging from 0 to 3 was applied to each observed bronchus, with approximately a total of 10 areas being scored (30).

Cell culture

A mouse macrophage line (MH-S), human airway epithelial cell line (NCI-H292), and human fibroblast cell line (WI-38) were obtained from American Type Culture Collection (American Type Culture Collection, Manassas, VA) and were cultured in 37°C at 5% CO_2 . Alveolar macrophages (AMs) were isolated from BAL fluid, as previously described (31). BAL fluids were centrifuged ($500 \times g$ for 5 min at 4°C), and BAL cells were resuspended with RPMI 1640 medium. The BAL cells were plated to a 24-well culture plate with a cover glass (Fisherbrand, Pittsburgh, PA). The cells were allowed to adhere for 2 h at 37°C under 5% CO_2 . After an initial study with several doses (10, 20, and 40 μ g/ml), we found that treatment of murine lung epithelial cells (MLE-12) with 40 μ g/ml HDM extracts increased the expression of STAT6 and phosphor-NF- κ B at 1, 4, and 6 h. In subsequent experiments, we treated the cells with 40 μ g HDM/ml in serum-free culture medium for 6 h.

Transfection, viral infection, and luciferase assay

AMs (MH-S) and human airway epithelial cell line (NCI-H292) cells were transfected with Lyn small interfering RNA (siRNA) (Lyn siRNA 20 μ M, Santa Cruz Biotechnology) with Lipofectamine 2000 according to the manufacturer's instructions. At 24 h after transfection, the transfected cells or cells treated with PP2 (Lyn inhibitor, 5 nM for 1 h) were stimulated by 40 μ g/ml HDM. We also used an adenoviral vector overexpressing con-

stitutive TGF- β 3 (and empty vector control) to study EMT in airway epithelial cells (H292). The adenoviral vector expressing TGF- β 3 was used at 10^9 particles on each well of a 24-well plate (1 d after seeded 100,000 H292 cells per well) and was kindly provided by Dr. D. Wang (Nanyang Technological University, Singapore, China) (32). We evaluated and found that the expression of TGF- β 3 was increased compared with the vector control after the TGF- β 3-viral infection; thereafter, we measured the EMT. For luciferase assay, a 3.7-kb segment of 5' flanking region of human MUC5AC gene (nucleotide from -3752/+7) was cloned into pGL3-Basic Luciferase Vector (Promega, Madison, WI) (33, 34). A PLR-TK vector was used as a control plasmid to measure transfection efficiency. Human airway epithelial cells were seeded in 24-well tissue culture plates, and cell transfection was performed using Lipofectamine 2000 according to the manufacturer's instructions. The transfected 16HBE cells were stimulated with 100 pg/ml for 48 h. Luciferase activity was measured according to the manufacturer's instruction (Promega).

Immunofluorescent staining and confocal microscopy

Frozen lung tissues at -80°C were sectioned, and immunohistochemistry staining was performed on glass slides, using standard histological methods. The sections were fixed in acetone and blocked at room temperature. Tissue sections were incubated with α -SMA, MUC5AC, and collagen type 1 Ab, respectively (Santa Cruz Biotechnology). A mouse isotype serum instead of the primary Abs was used as a negative control. FITC-conjugated goat anti-rabbit Abs or tetramethylrhodamine isothiocyanate-conjugated anti-mouse secondary Abs were used to probe the primary Abs. Tissue sections were viewed with a Zeiss META 510 confocal microscope (Zeiss). The airway remodeling parameters were analyzed by previously described methods (35). The fluorescence intensity was calculated as integrated fluorescence intensity per micrometer of airway basement membrane and then normalized to negative control values (in 10 randomly selected airway inflam-

matory fields or 100 randomly chosen airway cells) (14). Primary macrophages were immunostained for TGF- β 3. Human airway epithelial cells were immunostained for fibronectin and E-cadherin. Human fibroblasts were immunostained for collagen I. Tetramethylrhodamine isothiocyanate-conjugated anti-rabbit secondary Abs or FITC-conjugated anti-mouse secondary Abs were used to probe the primary Abs. The cell nucleus was stained with DAPI reagents.

RT-PCR

Total RNA was extracted from lung tissue with TRIzol Reagent (Invitrogen Life Technologies, Carlsbad, CA) as recommended by the manufacturer. RNA was resuspended in 50 μL nuclease-free water, and its concentration was determined using a Nanodrop machine (Epoch, BioTek). The RNA samples were kept at -80°C until use. Semiquantitative RT-PCR primers for MUC5AC, collagen I, and collagen III were designed as described previously (36, 37). PCR primers for collagen I were 5'-TGTTCTGGTTCCTCAGGGTAG-3' (forward) and 5'-TTGTCGTAGCAGGGTCTTTC-3' (reverse). PCR primers for collagen III were 5'-TGCCACAGCCTTCTACACCT-3' (forward) and 5'-CCAGCTGGGCCTTTGATACCT-3' (reverse). PCR primers for MUC5AC were 5'-GGACCAAGTGGTTTGACACTGAC-3' (forward) and 5'-CCTC-ATAGTTGAGGCACATCCCAG-3' (reverse). PCR primers for GAPDH were 5'-TCAACGGCACAGTCAAGG-3' (forward) and 5'-ACCAGTGGATGC-AGGGA-3' (reverse). Reverse transcription was performed using 1.5 μg RNA and avian myeloblastosis virus reverse transcriptase. The relative expression levels in each sample were quantified using GAPDH as an internal loading reference.

Western blotting and immunoprecipitation

Lung tissues or cells were homogenized in radioimmunoprecipitation assay (RIPA) buffer for dot blotting and Western blotting analysis with an optional protease inhibitor mixture (Roche or Fisher Scientific). Cultured cells were

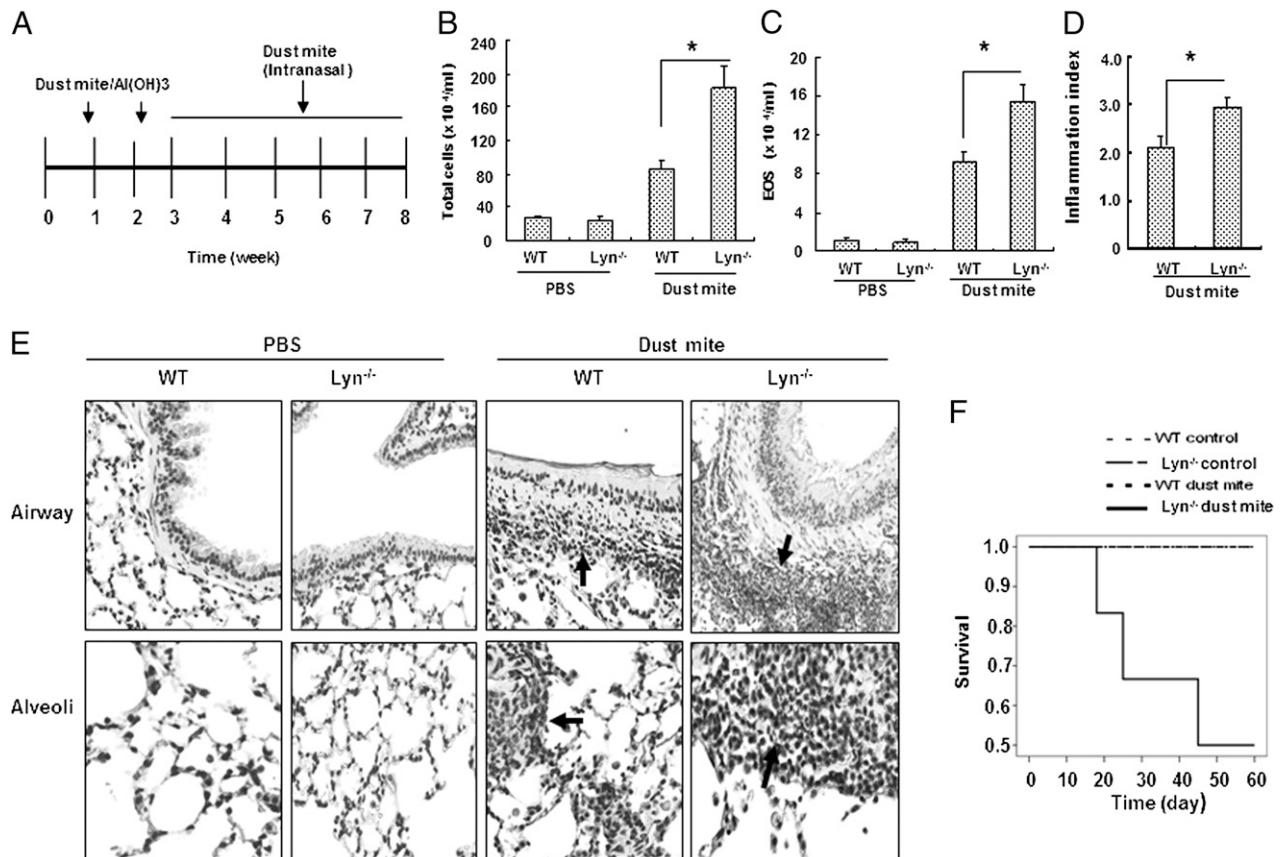


FIGURE 1. Chronic allergen exposure increased inflammation and decreased survival in $\text{Lyn}^{-/-}$ mice. **(A)** Sensitization and challenge protocol for $\text{Lyn}^{-/-}$ and WT mice with HDM ($n = 8$ mice for each group). **(B and C)** The number of total cells and eosinophils (Eos) in the BAL of mice exposed to PBS or HDM, as determined by differential cell analysis. **(D)** Inflammatory cell infiltration was determined in the lungs of $\text{Lyn}^{-/-}$ mice, allergen-challenged WT mice, and sham-control mice in **(E)** (10 random areas). **(E)** Lung tissues were stained with H&E (arrows indicating typical inflammatory regions with inflammatory cells; original magnification, $\times 200$). Data are the mean $\pm$ SEM and are representative of eight mice evaluated in each group (one-way ANOVA [Tukey post hoc]; $*p < 0.05$, $**p < 0.01$). **(F)** Survival curves of $\text{Lyn}^{-/-}$ and WT mice after chronic exposure to HDM, using Kaplan-Meier methods ($n = 8$). $*p < 0.01$.

also lysed with RIPA buffer, and protein concentrations were measured using a Bio-Rad assay. For Western blotting, lysates (20 μ g) were resolved by NaDodSO₄ PAGE (SDS-PAGE, 10%) at 100 V for 2 h and transferred to microporous polyvinylidene difluoride membrane at 100 mA for 2 h. The membranes were blotted with ERK1/2, TGF- β 1, TGF- β 3, SHIP1, NF- κ B, STAT6, Lyn, Smad, β -actin, phospho-Smad phospho-ERK, phospho-NF- κ B, phospho-STAT6 (1:1000 dilution) (Santa Cruz Biotechnology). The catalog numbers of the Abs were TGF- β 1 (clone no.: V; cat. no.: sc-146), TGF- β 3 (clone no.: III; cat. no.: sc-83), IL-4 (clone no.: H-129; cat. no.: sc-7919), NF- κ B (clone no.: F-6; cat. no.: sc-8008), STAT6 (clone no.: S-20; cat. no.: sc-621), Lyn (clone no.: 44; cat. no.: sc-15), Smad2/3 (clone no.: H-2; cat. no.: sc-7960), β -actin (clone no.: N-21; cat. no.: sc-130656), phospho-Smad (clone no.: pThr²²⁰; cat. no.: sc-SAB4300252), phospho-NF- κ B (clone no.: Ser³³⁷; cat. no.: sc-330220-R), phospho-STAT6 (clone

no.: Tyr⁶⁴¹; cat. no.: sc-11762). For immunoprecipitation, cells were lysed in RIPA lysis buffer. Protein A/G-agarose beads (Pierce) were used to precipitate Ab-Ag complexes and were washed in PBS. The beads were boiled in SDS sample buffer for 5 min and analyzed by SDS-PAGE and immunoblotting with specific Abs (20). HRP-conjugated goat anti-rabbit Abs (1:4000) or HRP-conjugated anti-goat secondary Abs (1:8000) or HRP-conjugated anti-mouse secondary Abs (1:2000) were used to bind primary Abs and visualized via an ECL kit (Pierce). The band intensity was evaluated against the loading control to derive a ratio value using Quantity One software (Bio-Rad). To gauge the interaction between Lyn domains and TGF- β 3, GST peptides containing various Lyn functional domains [Src homology (SH) 2, SH3, SH2/SH3, and kinase domain] were prepared as we previously described (14) and incubated with H292 cell lysates after HDM exposure and pulled down by GST Abs. The complexes

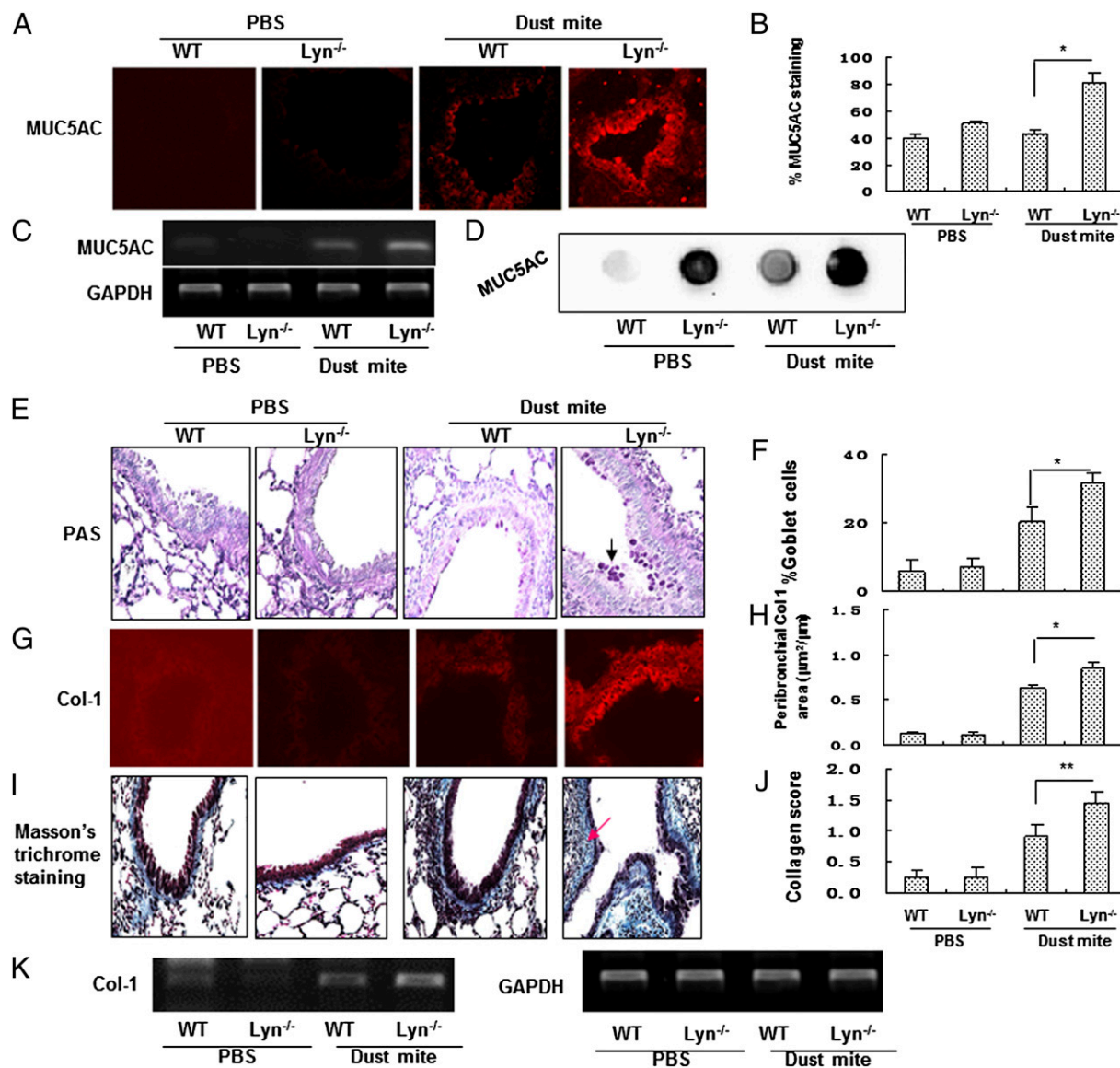


FIGURE 2. Chronic allergen exposure induced mucus hypersecretion and peribronchial collagen deposit in Lyn^{-/-} mice. **(A)** Immunohistochemistry of MUC5AC in the lungs of WT and Lyn^{-/-} mice exposed to HDM or PBS (merged images). Arrow shows MUC5AC⁺ cells in airway in (A) (the markers similarly indicated below). Original magnification $\times 200$. **(B)** Percentages of cells staining positive for MUC5AC in (A). Positive cells were counted in 10 random airway epithelial cell fields and presented as the mean $\pm$ SEM. * p < 0.01. **(C)** MUC5AC mRNA expression was determined in the lungs of Lyn^{-/-} mice and WT mice using RT-PCR. GAPDH was measured for internal controls. **(D)** MUC5AC protein was also determined in Lyn^{-/-} mice and WT mice using ELISPOT assay. **(E)** PAS staining of epithelial goblet cells in the lungs of WT and Lyn^{-/-} mice exposed to HDM or PBS. **(F)** Quantification of PAS⁺ cell percentage in 10 random airway epithelial cell fields in (E) (original magnification, $\times 200$, * p < 0.01). **(G)** Immunohistochemistry of collagen type I (Col I) in the lungs of WT and Lyn^{-/-} mice exposed to HDM or PBS. **(H)** The fluorescent intensity per micrometer of peribronchial collagen type I in 10 random basement membrane fields in (G) and presented as the mean $\pm$ SEM. Original magnification $\times 200$. * p < 0.01. **(I)** Masson's trichrome staining of collagen in the lungs of WT and Lyn^{-/-} mice. **(J)** Quantitation of Masson's trichrome staining of the lungs of WT and Lyn^{-/-} mice in (I) (original magnification, $\times 200$, ** p < 0.05). **(K)** mRNA of collagen type I and collagen type III was examined in HDM Lyn^{-/-} mice and HDM WT mice by RT-PCR. Data are representative of eight mice evaluated in each group (one-way ANOVA [Tukey post hoc]).

were then detected with anti-TGF- β 3 Abs for evaluating protein–protein interactions.

Statistical analysis

All experiments were performed at least three times in triplicate. The results were expressed as the mean $\pm$ SD. Statistical analysis was performed by one-way ANOVA with Tukey post hoc tests or Student *t* tests, and the significance level between two groups was defined as $p < 0.05$. The survival was determined using Kaplan–Meier analysis. The data were analyzed by SPSS 10.0 statistical software.

Results

Lyn^{−/−} mice showed increased inflammation following repetitive HDM challenge

To investigate the role of Lyn in chronic airway inflammation, we used initial sensitizations by i.p. injection of HDM, followed with repetitive intranasal instillation to induce chronic airway inflammation in *Lyn*^{−/−} mice and WT mice (Fig. 1A). These HDM challenges resulted in an $\sim$ 2-fold increase in total inflammatory cells and a 1.6-fold increase in eosinophils in the lungs of *Lyn*^{−/−} mice compared with WT mice ($p < 0.01$) (Fig. 1B, 1C), along with an increased inflammation index (Fig. 1D). Histological alterations reflecting inflammatory responses were also observed in the lungs and airways in HDM-challenged *Lyn*^{−/−} mice and WT mice compared with sham-challenged controls (Fig. 1E). Furthermore, inflammatory cell infiltration in the airway, around blood vessels, and in alveoli increased markedly in *Lyn*^{−/−} mice compared with WT mice (Fig. 1E). We have also noted significant airway smooth muscle thickening and angiogenesis in *Lyn*^{−/−} mice compared

with WT mice. In addition, survival was significantly shortened in *Lyn*^{−/−} mice compared with WT mice, using Kaplan–Meier analysis (Fig. 1F, $p < 0.01$). As the unchallenged *Lyn*^{−/−} mice did not have a precedent of mortality, the increased death rates appeared to be directly associated with the allergen challenge. The precise mechanism for inducing mouse fatality remains to be determined.

Lyn^{−/−} mice had increased mucus secretion following repetitive HDM challenge

Previous studies showed that chronic HDM exposure caused remodeling and Lyn deficiency exacerbated the eosinophilic inflammatory response (8, 24, 38). However, the effect of Lyn deficiency on chronic airway remodeling remains unclear. Our studies found that chronic HDM exposure caused vigorous mucus secretion in WT mice and *Lyn*^{−/−} mice (Fig. 2). MUC5AC immunostaining (Fig. 2A, 2B) demonstrated a 2-fold increase in the airway of HDM-challenged *Lyn*^{−/−} mice compared with that of WT mice. The number of MUC5AC⁺ epithelial cells and goblet cells also increased $\sim$ 1.5-fold in HDM-exposed *Lyn*^{−/−} mice compared with that in WT mice ($p < 0.01$).

MUC5AC mRNA levels also increased in HDM-challenged *Lyn*^{−/−} mice compared with both HDM WT mice and sham WT mice (Fig. 2C). Furthermore, ELISPOT assay revealed an increase of MUC5AC protein in *Lyn*^{−/−} mice compared with that in WT mice (Fig. 2D). To further evaluate the accelerated mucus production, we performed PAS staining and found a 1.9-fold increase of mucus secretion in the airway of HDM-challenged *Lyn*^{−/−} mice compared with that of WT mice (Fig. 2E, 2F).

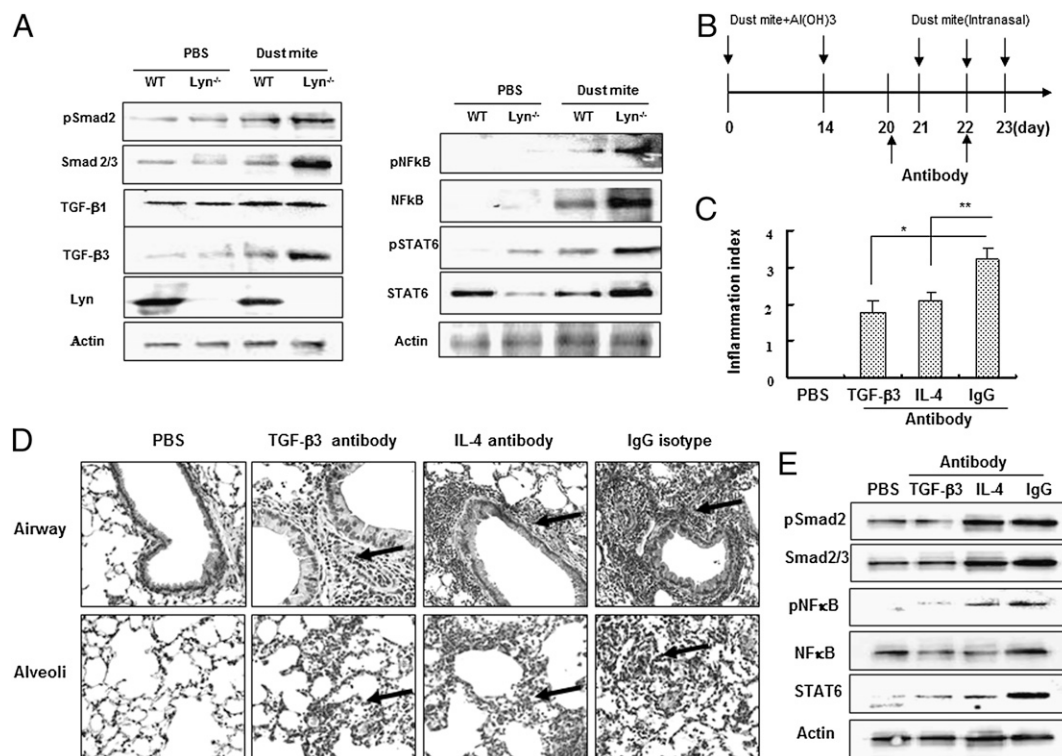


FIGURE 3. Lyn deficiency upregulated STAT6, NF- κ B, and TGF- β 3/Smad. (A) Western blot analysis of TGF- β 3, TGF- β 1, Smad2/3, STAT6, and NF- κ B as well as phosphorylation of STAT6, NF- κ B, and Smad2 in *Lyn*^{−/−} and WT mouse lungs repetitively exposed to HDM or PBS. β -actin was used as loading control (representative data of eight mice evaluated in each group). (B) Protocol for sensitization and challenge of *Lyn*^{−/−} mice and WT mice with dust mite and neutralizing Abs ($n = 5$). (C) Inflammation index was estimated from 10 random fields with airway tissue inflammation (one-way ANOVA [Tukey post hoc]; * $p < 0.05$, ** $p < 0.01$). (D) H&E staining of the lung in *Lyn*^{−/−} mice after neutralizing Ab treatment and exposure to dust mite. Arrows show tissue remodeling and inflammation ($n = 5$). Original magnification $\times 200$. (E) Expression of NF- κ B, Smad2/3, and STAT6, and phosphorylation of NF- κ B and Smad2 in the lung of *Lyn*^{−/−} mice. After neutralizing Ab treatment, mice were exposed to dust mite ($n = 5$). Data are representative of five mice evaluated in each group.

Lyn^{-/-} mice showed increased peribronchial collagen deposition following repetitive HDM challenge

Peribronchial collagen deposition is a well-recognized feature of airway remodeling. Using immunostaining, we found that the expression of peribronchial collagen type 1 increased ~1.6-fold in HDM-exposed *Lyn*^{-/-} mice compared with WT mice ($p < 0.01$) (Fig. 2G, 2H). Furthermore, Masson's trichrome staining showed that chronic exposure to HDM increased peribronchial collagen deposition in WT mice and *Lyn*^{-/-} mice; and, yet, peribronchial collagen deposition increased ~1.8-fold in HDM-exposed *Lyn*^{-/-} mice compared with WT mice (Fig. 2I, 2J). In addition, mRNA levels of collagen I increased in HDM-exposed *Lyn*^{-/-} mice compared with HDM-exposed WT mice or sham WT mice (Fig. 2K).

Lyn deficiency upregulated TGF- β 3/Smad and STAT6 during HDM challenge

To gain insight into the underlying molecular mechanism of chronic inflammation, we set out to define the role of the TGF- β /Smad pathway in this model. Fig. 3A shows that HDM-challenged *Lyn*^{-/-} mice displayed increased protein expression of Smad2/3, STAT6, and NF- κ B as well as phosphorylation of Smad2, STAT6, and NF- κ B compared with HDM-exposed WT mice. Previous studies have

established that STAT6 is critical for the development of airway remodeling (39). Chronic exposure to HDM increased the expression of TGF- β 1 in WT mice and *Lyn*^{-/-} mice. However, TGF- β 3 (but not TGF- β 1) increased by 3-fold in HDM-exposed *Lyn*^{-/-} mice compared with HDM-exposed WT mice (Fig. 3A). The protein levels of STAT6, NF- κ B, and particularly Smad2/3 (3-fold) increased in HDM-exposed *Lyn*^{-/-} mice as quantified by densitometry analysis (Supplemental Fig. 1A and B, $p < 0.05$).

*Neutralization of TGF- β 3 significantly inhibited airway inflammation in *Lyn*^{-/-} mice*

To further characterize the role of TGF- β 3 in airway remodeling, we i.p. administered TGF- β 3 neutralizing Abs to *Lyn*^{-/-} mice and evaluated the airway inflammation (Fig. 3B). As previous reports showed that IL-4 neutralizing Abs can alleviate asthmatic intensity, we also used IL-4 neutralizing Abs for comparative analysis. Neutralizing effects through i.p. injection of Abs was previously achieved in suppressing airway inflammation (4). Both TGF- β 3 and IL-4 neutralizing Abs, compared with isotype IgG Abs, decreased the infiltration of inflammatory cells in the lungs of *Lyn*^{-/-} mice (Fig. 3C, 3D). Importantly, TGF- β 3 neutralizing Abs diminished airway inflammation to a significantly greater extent than did IL-4 neutralizing Abs. TGF- β 3 neutralizing Abs

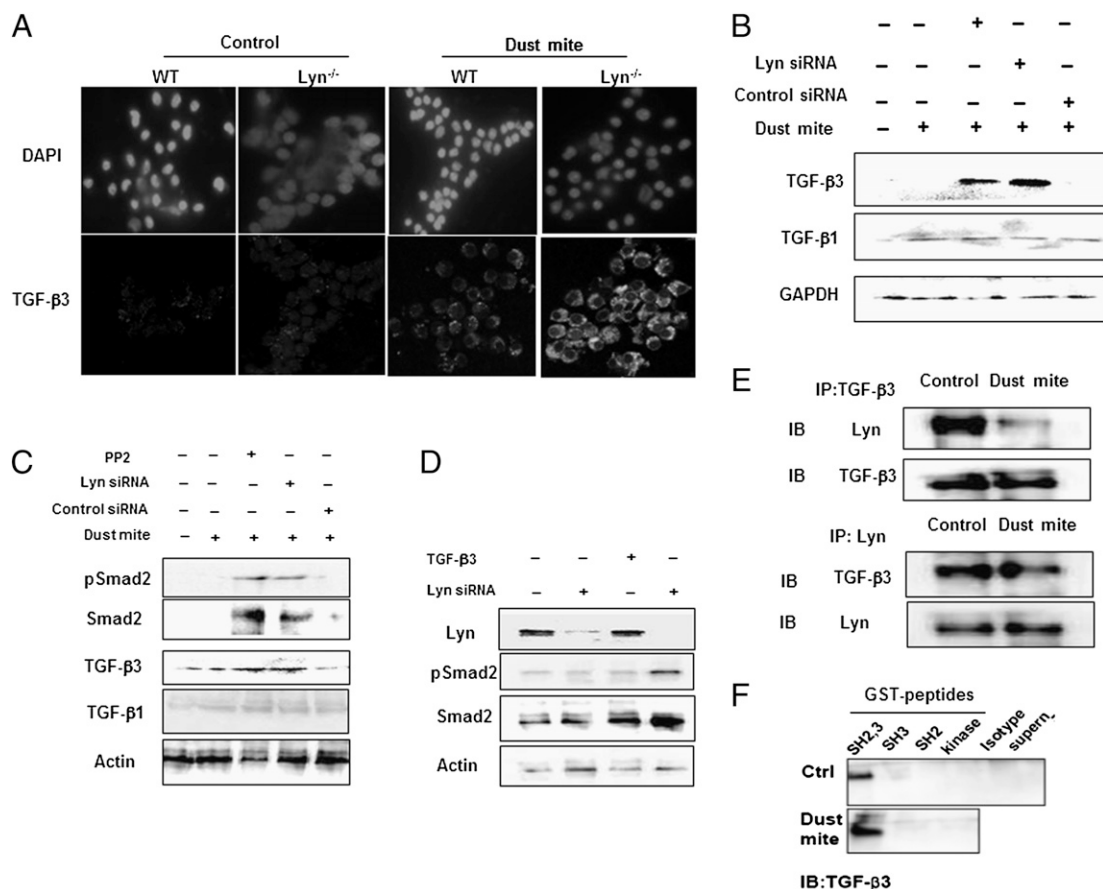


FIGURE 4. Lyn deficiency or knockdown enhances TGF- β 3 in HDM-exposed macrophage cells and airway epithelial cells. **(A)** Primary AMs were isolated from *Lyn*^{-/-} mice and WT mice (representative data of five mice evaluated in each group), and immunostaining of TGF- β 3 in the cells was done after stimulation with 20 μ g/ml HDM for 6 h (original magnification $\times 1000$). Arrows show TGF- β 3⁺ cells. **(B)** Lyn siRNA transfection and Lyn inhibitor (PP2) treatment increased TGF- β 3 in mouse macrophage MH-S cells in the presence of 20 μ g/ml HDM. **(C)** Lyn siRNA and Lyn inhibitor (PP2) increased TGF- β 3, but not TGF- β 1, in an airway epithelial cell line (H292) in the presence of 20 μ g/ml HDM. HDM also increased Smad2/3 expression and Smad2 phosphorylation. **(D)** TGF- β 3 increased Smad2/3 expression and Smad2 phosphorylation in a Lyn siRNA-transfected airway epithelial cell line. **(E)** Coimmunoprecipitation determined the interaction between Lyn and TGF- β 3. TGF- β 3 and Lyn Abs were used to precipitate TGF- β 3 and Lyn complexes, respectively. **(F)** GST peptides containing various Lyn functional domains were incubated with H292 cell lysates after HDM exposure and pulled down by GST Abs. The complexes were then detected with anti-TGF- β 3 Abs. Data are representative of three experiments.

also decreased the expression of Smad, NF- κ B, and STAT6, as well as the phosphorylation of Smad and NF- κ B (Fig. 3E). Although we have not used the previously reported TGF- β 3 neutralizing Abs (clone 20724; R&D Systems) (40), our data showed that the neutralizing ability of the Abs (sc166861; Santa Cruz Biotechnology) that were also raised based on C-terminal sequences can block inflammatory cytokine secretion (data not shown) in addition to the blockade of airway remodeling noted in *Lyn*^{-/-} mice. IL-4 neutralizing Abs decreased the expression and phosphorylation of NF- κ B and STAT6, but did not inhibit expression and phosphorylation of Smad (Fig. 3E, Supplemental Fig. 1C). These data indicate that increased TGF- β 3 may have a detrimental role in chronic airway inflammation. To demonstrate whether *Lyn* deficiency contributed to the enhanced expression of TGF- β 3 in AMs, we examined TGF- β 3 in primary AMs from WT and *Lyn*^{-/-} mice and found that *Lyn* deficiency significantly increased TGF- β 3 expression (Fig. 4A, Supplemental Fig. 2A). Collectively, these data suggest a role for *Lyn* in the negative regulation of TGF- β 3 in airway remodeling.

HDM induced TGF- β 3 in *Lyn* knockdown cells

To further evaluate the role of *Lyn* in HDM-induced airway remodeling, we investigated the role of *Lyn* siRNA and *Lyn* inhibitor (PP2) in TGF- β 3 expression. We found that inhibition of *Lyn* enhanced the expression of TGF- β 3 in a mouse AM cell line (MH-S) after exposure to HDM (Fig. 4B, Supplemental Fig. 2B).

However, no comparable enhancement was found for TGF- β 1 in an identical condition. Furthermore, *Lyn* siRNA and *Lyn* inhibitor (PP2) also enhanced TGF- β 3 expression in human airway epithelial cell lines following HDM challenge, which also increased Smad2/3 expression and Smad2 phosphorylation (Fig. 4C). *Lyn*-deficient mice showed a significant increase in TGF- β 3, which may contribute to a significant increase in Smad expression and phosphorylation (Fig. 4D, Supplemental Fig. 2C, $p < 0.05$). To examine a potential interaction between *Lyn* and TGF- β 3, coimmunoprecipitation was performed. Our preliminary analysis indicates that an interaction took place between *Lyn* and TGF- β 3, which was decreased in cells exposed to HDM (Fig. 4E, Supplemental Fig. 2D). These data suggest that *Lyn* may inhibit TGF- β 3 through direct binding. Next, we analyzed the domain of *Lyn* involved in this interaction, using *Lyn*-GST peptides, and the results showed that both Src homolog domains SH2 and SH3 were required for protein-protein interaction between *Lyn* and TGF- β 3 (Fig. 4F). Although further evidence is needed to define the physiologically relevant interaction, these results indicate that *Lyn* may regulate TGF- β 3 via protein interaction with TGF- β 3.

Role of TGF- β 3 in EMT, collagen, and MUC5AC expression

Because previous studies indicate that α -SMA may deposit in a particular site during airway remodeling, we examined the levels and localization of α -SMA in our model. We found that peri-

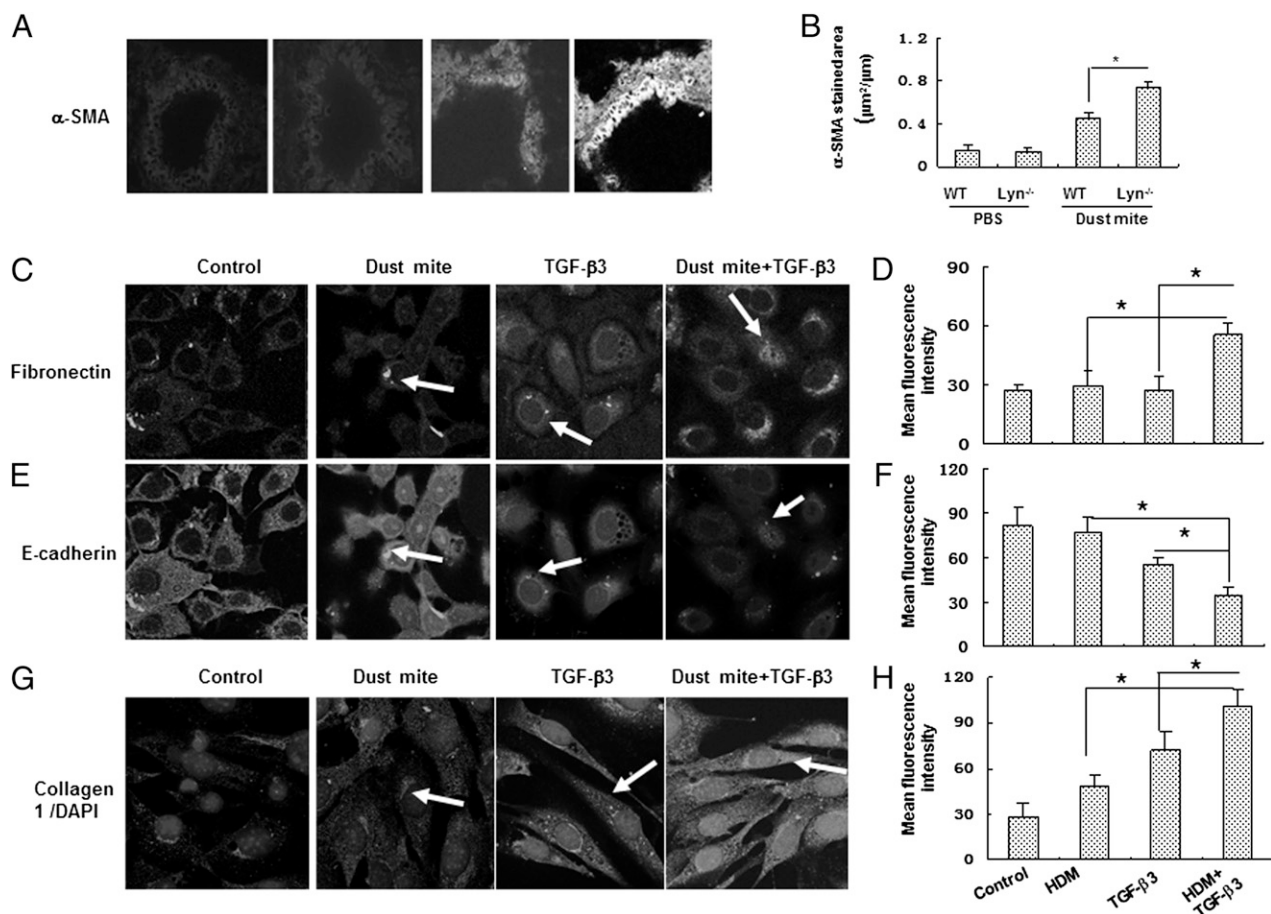


FIGURE 5. TGF- β 3 role in EMT, collagen I, and MUC5AC expression in human epithelial cells and fibroblasts as well as in the lung. **(A)** Immunohistochemistry of α -SMA in the lung of WT and *Lyn*^{-/-} mice exposed to HDM or PBS. **(B)** The fluorescence intensity per micrometer of α -SMA in 10 random fields in **(A)** [original magnification in **(A)**, $\times 630$]. Data are representative of eight mice evaluated in each group (one-way ANOVA [Tukey post hoc]; $*p < 0.01$). Immunohistochemistry of fibronectin (green, **C** and **D**), E-cadherin (red, **E** and **F**), and collagen I (red for Col-I and blue for DAPI, **G** and **H**) in airway epithelial cells, which were stimulated with HDM and TGF- β 3 (original magnification $\times 400$). Arrows indicate relevant markers in positively stained cells. Data are representative of three experiments (one-way ANOVA [Tukey post hoc]; $*p < 0.01$).

bronchial areas with α -SMA stain increased in HDM-exposed WT and Lyn^{-/-} mice compared with sham-exposed WT mice and Lyn^{-/-} mice. However, HDM exposure further increased α -SMA by 1.9-fold in Lyn^{-/-} mice compared with that in WT mice (Fig. 5A, 5B). Previous studies suggest that EMT is a significant contributor to airway wall thickening in the pathological course of severe asthma (5) and that HDM exposure promoted EMT in human bronchial epithelium (41). Although autocrine TGF- β 3 induced EMT in lung cancer cell development as well as stem cell maintenance (42), the role of TGF- β 3 in EMT in airway remodeling has not been reported. We found that neither HDM nor TGF- β 3 peptide (5 ng/ml; PeproTech cat. no.: AF-100 36E) alone could increase fibronectin expression by any significant margin (Fig. 5C, 5D). In contrast, HDM challenge plus TGF- β 3 treatment significantly inhibited E-cadherin expression along with increased fibronectin, indicating the induction of EMT (Fig. 5E, 5F). TGF- β 3 plus HDM challenge also increased collagen I expression in human fibroblasts (Fig. 5G, 5H). These results confirmed the critical role of TGF- β 3 in inducing EMT and inflammatory outcomes via the combined use of TGF- β 3 peptide and HDM.

TGF- β 3 adenovirus plus HDM induced significant EMT in the airway epithelial cells

To further validate the results, we studied EMT in human H292 airway epithelial cells, using TGF- β 3 expression adenovirus (provided by Dr. D. Wang, Nanyang Technological University, Singapore, China) (32). According to the existing literature, we infected H292 cells with the TGF- β 3 adenovirus (10^9 viral particles per well) and found significantly increased TGF- β 3 compared with that in groups treated by the control vector, indicating that endogenous TGF- β 3 levels cannot initiate alterations in the epithelium. We next examined the effect of HDM on EMT in TGF- β 3 adenovirus-infected cells and found that TGF- β 3 adenovirus plus HDM induced significant EMT in airway epithelial

cells, compared the vector control, with increased fibronectin but decreased E-cadherin (Fig. 6A, 6B, $p < 0.05$).

To further characterize the role of Lyn in regulating MUC5AC expression, we cloned a 3.7-kb segment of 5' flanking region of human MUC5AC into pGL3 luciferase vector (Promega,). By measuring the luciferase activity in cultured human airway epithelial cells, HDM significantly induced MUC5AC gene expression compared with sham controls ($p < 0.001$). Furthermore, Lyn knockdown in epithelial cells significantly increased HDM-induced MUC5AC gene expression compared with that in control cells ($p < 0.001$) (Fig. 6C). Finally, we also examined EMT transition in HDM-challenged lungs and found that significant EMT occurred in Lyn^{-/-} mice versus WT mice (data not shown).

Asthma patients exhibited decreased Lyn and increased TGF- β 3/ Smad2/3 expression in airway tissue

To evaluate the clinical relevance of the animal and cellular studies, we measured the expression of Lyn, TGF- β 3, and Smad2/3 in airway tissue from normal individuals or from chronic asthma patients collected by bronchoscopy. Asthmatic patients exhibited increased chronic airway inflammation and MUC5AC (Fig. 7A, 7B, $n = 7$). In addition, the expression of Lyn decreased ~ 3.5 -fold in the airway of asthma patients compared with that of normal individuals, whereas the expression of TGF- β 3 increased ~ 3.1 -fold, as determined by quantitative immunostaining (Fig. 7C; differential interference contrast is used to indicate the tissue structure). Similar to TGF- β 3, Smad2/3 expression and Smad2 phosphorylation also significantly increased in the airway of asthma patients compared with that of normal individuals (Fig. 7D, right panels, $p < 0.05$).

Discussion

This study reports that Lyn^{-/-} mice manifested decreased survival and increased airway inflammation and airway structural alterations following repetitive exposure to HDM (Fig. 1). Specifically,

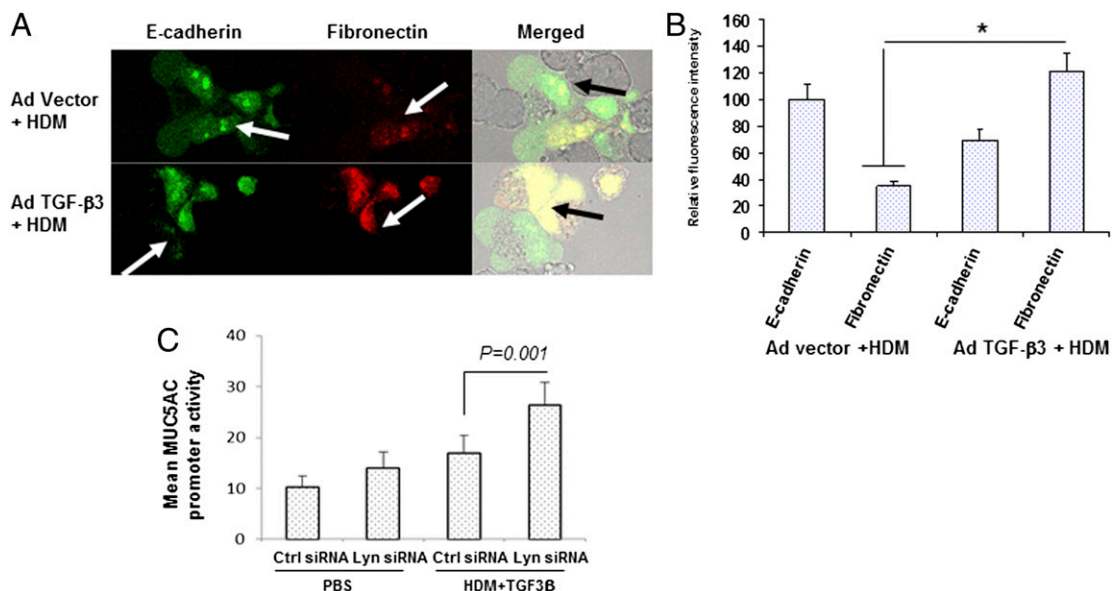


FIGURE 6. Adenoviral TGF- β 3 infection increased HDM-induced EMT in airway epithelial cells. **(A)** Adenoviral TGF- β 3 infection (10^9 particles per well) increased HDM-induced EMT in airway epithelial cells (H292) determined by immunocytochemistry staining of fibronectin and E-cadherin. **(B)** Quantification of (A) by ImageJ software (original magnification $\times 100$). Arrows indicate relevant markers in positively stained cells. Although E-cadherin was not significantly reduced, fibronectin was dramatically increased by HDM treatment in TGF- β 3 adenoviral-infected cells (one-way ANOVA [Tukey post hoc]; $*p < 0.05$). **(C)** MUC5AC activation by determined luciferase promoter assay. The human airway epithelial H292 cells were cotransfected with MUC5AC promoter luciferase plasmid and Lyn siRNA versus control vector and scrambled siRNA. The cells were then stimulated with HDM and 100 pg/ml TGF- β 3 for 48 h. Luciferase activity was measured. Data are representative of three experiments in triplicate and expressed as the mean $\pm$ SEM (one-way ANOVA [Tukey post hoc]; $*p < 0.05$, $**p < 0.01$).

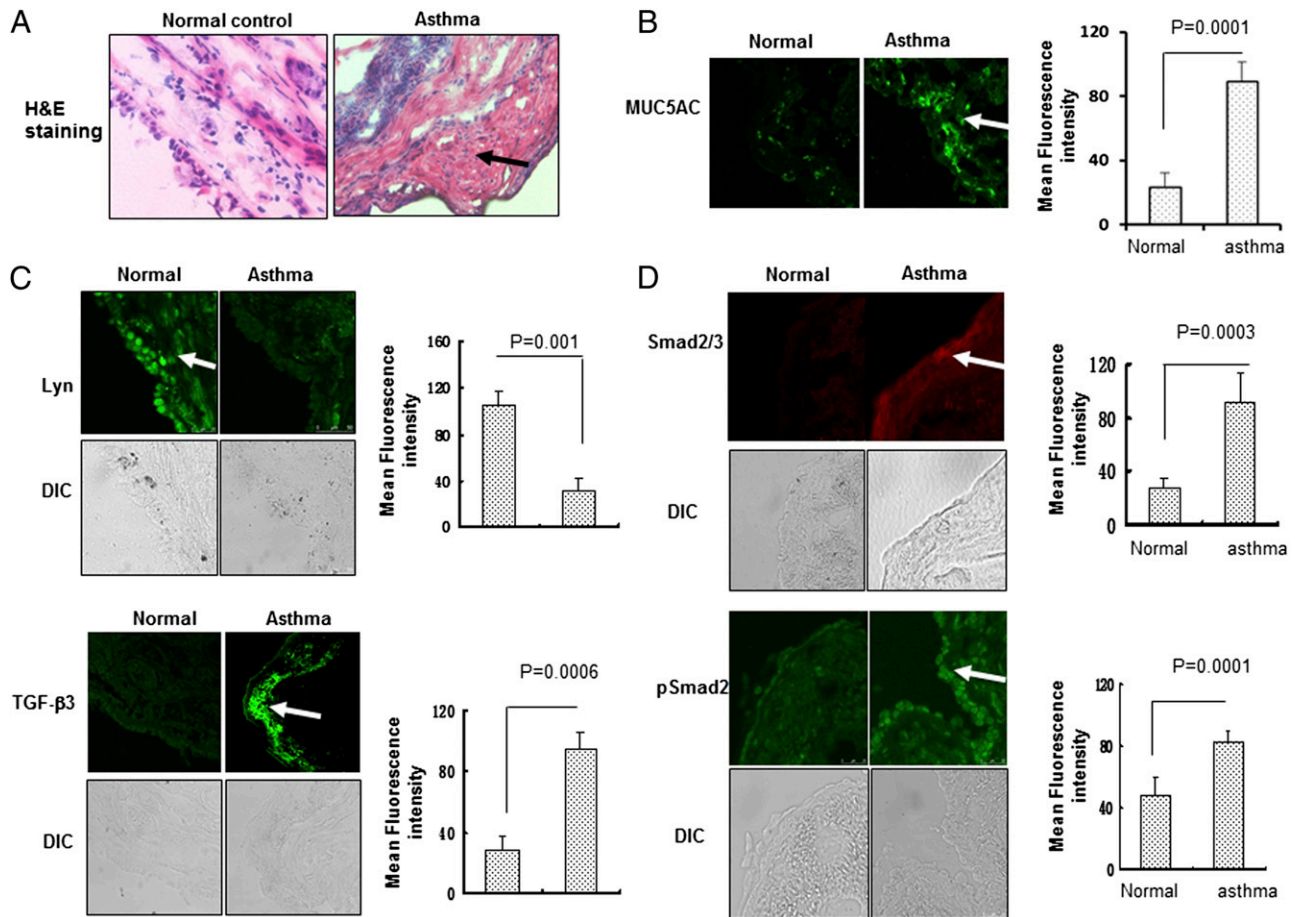


FIGURE 7. Asthma exhibited decreased Lyn and increased TGF- β 3/Smad2/3 expression in airway tissue. Lyn, TGF- β 3, Smad2/3, and Smad2 phosphorylation levels in normal airway tissue and in airway tissue from asthma patients. **(A)** H&E staining of normal airway tissue and airway tissue from asthma patients. **(B)** Immunostaining for the expression of MUC5AC. **(C)** Immunostaining for the expression of Lyn, TGF- β 3. **(D)** Immunostaining for the expression of Smad2/3 and the phosphorylation of Smad2. The fluorescence intensity was quantified per micrometer of α -SMA in 10 random fields, using ImageJ software (original magnification, $\times 630$). Arrows indicate staining compared with the normal controls. Data are the mean $\pm$ SEM and representative of seven patients (three male and four female) and seven normal subjects (four male and three female) evaluated (one-way ANOVA [Tukey post hoc]). Ages of patients (years, mean $\pm$ SD), 34.4 ± 12.1 ; normal subjects, 31 ± 14.1 . Forced expiratory volume in one second (FEV1%) in patients, 60 ± 8.1 ; normal subjects, 82 ± 10.2 ($p < 0.001$).

HDM-challenged Lyn^{-/-} mice exhibited elevated inflammatory cell infiltration, mucus production, collagen deposition in the airway as well as increased α -SMA expression, and thickness of the smooth muscle layer. In addition, HDM challenge shortened lifespans of Lyn^{-/-} knockout mice versus those of WT mice, which may be related to increased airway inflammation and airway remodeling. Therefore, these data suggest that Lyn may negatively regulate airway inflammation as well as airway remodeling.

In lung tissue, collagen I is the matrix to provide resistance to tensile stresses. Deposition of collagens is also a major feature of airway remodeling in chronic asthma (43). Changes in the extracellular matrix and increased airway smooth muscle mass are also major contributors to airway remodeling in asthma (44). We found that HDM challenges also increased markedly the protein and mRNA of collagen I in Lyn^{-/-} mice. Furthermore, HDM challenges significantly increased the thickness of the peribronchial smooth muscle layer and expression of α -SMA in Lyn^{-/-} mice relative to WT mice. These results suggest that Lyn deficiency aggravates airway remodeling, with increased mucus production and collagen deposition.

Lyn is primarily a BCR regulator and is expressed in a variety of cells, including T cells (25). IL-4⁺ cells along with IgE levels (in

relative light units) in spleen CD4⁺ cells significantly increased in HDM-challenged Lyn^{-/-} mice (Supplemental Fig. 3A, 3C). We found that Lyn deficiency also affected IL-4 secretion by T cells and TNF- α (but not IL-10) in the lung (Supplemental Fig. 3B, 3D). These results suggest that Lyn may serve as a negative regulator in allergen-induced IL-4 and TNF- α . HDM-specific IgE in allergen-treated WT mice was increased against the PBS control, but was less significantly increased than Lyn^{-/-} mice (Supplemental Fig. 3C), indicating the critical role of Lyn in chronic allergic airway inflammation. In addition, the IL-4/STAT6 axis plays a key part in asthmatic airway remodeling (44–47). NF- κ B is responsible for the transcription of many proinflammatory genes, increasing the production of cytokines, adhesion molecules, and chemokines (48). TGF- β was also involved in inducing in vitro differentiation of myofibroblasts and collagen production through a Smad2-dependent mechanism. Anti-TGF- β Abs showed an inhibition of airway remodeling (49). Lyn contributes to the upregulation of Ras-ERK1/2 and PI3K-Akt cascades. Lyn knockdown completely abolished ERK1/2 phosphorylation (50). Lyn attenuated NF- κ B signaling, and upregulated Lyn exhibited weak NF- κ B activation (51). We showed that Lyn deficiency increased the expression of Smad2, STAT6, and NF- κ B as well as the phosphorylation of Smad2, STAT6, and NF- κ B. Strikingly,

a specific increase in TGF- β 3 was noted in HDM-challenged Lyn^{-/-} mice compared with WT mice, whereas TGF- β 1 or TGF- β 2 expression had no significant increase. We assume that TGF- β 3 can affect Smad and STAT6 levels, which in turn regulates cytokine levels and inflammatory responses. In our other study, we found that Lyn deficiency upregulated STAT6 in IL-4- or IL-13-stimulated 16HBE cells. Thus, besides the direct effect, Lyn may also downregulate IL-4 and IL-13 to inhibit STAT6 and Smad2, which led to lower NF- κ B nuclear translocation, as we have found in other studies. We also speculated that TGF- β 3 can indirectly affect the levels of STAT6 and NF- κ B. Lyn deficiency did not affect the expression of ERK1/2 and SHIP1; nor did it impact their phosphorylation. These results imply that Lyn specifically downregulates the TGF- β 3/Smad2, STAT6, and NF- κ B signals, whereas IL-4 and IL-13 may also alter STAT6 or NF- κ B levels.

Inhibition of IL-4 and IL-13 activities can suppress allergen-induced inflammation (52–54). Previous studies indicate that TGF- β 1 neutralization may alleviate airway remodeling (55), and anti-TNF- α treatment reduces mucous cell metaplasia in the mouse airway (56). In this study, we found that TGF- β 3 neutralizing Abs, specific for the C terminus between aa 345 and 375, decreased inflammatory cell infiltration in Lyn^{-/-} mice exposed to HDM. These data indicate that TGF- β 3 upregulates HDM-induced airway inflammation in Lyn^{-/-} mice, consistent with others' observations (57).

The previously reported neutralizing Abs (clone 20724; R&D Systems) and the currently used TGF- β 3 Abs (sc166861) are both raised against the C terminus of this molecule. Indeed, our data convincingly showed the neutralizing ability of the sc166861 Abs both in vitro and in vivo. Burton et al. (58) early used different Abs to characterize the cellular localization of TGF- β 3. Although antisera raised against the N terminus was predominantly immune reactive within epithelial cells, Abs specific for the C terminus reacted exclusively within the extracellular matrix underlying adjacent epithelia. This observation suggests that TGF- β 3 undergoes cleavage of a C-terminal fragment, which is then exported from cytosol to intercellular regions, which may be involved in EMT. The C-terminal fragment may form a mature portion of protein and is involved in intracellular signaling, highly relevant to our disease model. Owing to important biological activity, blocking aa 345–375 at the C terminus of TGF- β 3 affects the function of TGF- β 3.

TGF- β 3 is expressed in AMs, epithelial cells, and smooth muscle cells of vessels and bronchi of lungs (59). Our studies showed that AMs from Lyn^{-/-} mice exhibited increased TGF- β 3 following exposure to HDM, indicating that AMs may contribute to airway inflammation through TGF- β 3. We also observed that Lyn knockdown in human airway epithelial cells increased the expression of TGF- β 3 (but not TGF- β 1; Supplemental Fig. 4A, 4B) and that TGF- β 3 function was dependent on the Smad2 pathway. A preliminary analysis demonstrated that Lyn had a direct interaction with TGF- β 3, requiring both SH2 and SH3 domains. These data suggest that Lyn negatively regulates TGF- β 3 through protein–protein interactions, which warrants further investigation.

During EMT, lung cells lose epithelial properties and gain properties of mesenchymal cells (60). Downregulation of E-cadherin is a critical feature for EMT (32). We demonstrated that a combination of HDM and TGF- β 3 inhibited E-cadherin expression and enhanced fibronectin expression in airway epithelial cells, supporting an observation that airway fibroblasts from patients with severe asthma exhibit enhanced procollagen I (61). In addition, in cultured human epithelial cells, we confirmed that knockdown of Lyn resulted in increased MUC5AC, indicating that TGF- β 3 contributes to airway remodeling by promoting fibroblast growth, and that Lyn deficiency

enhances TGF- β 3-driven EMT and collagen I production in cultured cells. We have not found significantly altered expression for TGF- β 2 in Lyn-deficient mice. Furthermore, we used a human MUC5AC reporter gene assay and found that Lyn deficiency did not influence the levels of TGF- β 2 and mucus production. NCI-H292 is a human mucoepidermoid bronchiolar carcinoma cell line but is commonly used for studying airway remodeling in vitro. To ensure the verity of our data, we have also performed in vitro experiments with human airway epithelial cells (16HBE) and obtained similar results. Further, we confirmed most of our data in a normal murine alveolar epithelial cell line (MLE-12).

Importantly, we demonstrate that asthma patients with airway remodeling exhibited markedly increased TGF- β 3 and decreased Lyn. The data indicate that the Lyn–TGF- β 3 axis may play a key role in the development of clinical chronic airway inflammation/remodeling. Although a previous study showed that OVA induced a heightened Th2 response in Lyn-deficient mice, which is critically involved in dendritic cells (24), our study is, to our knowledge, the first to report a Lyn–TGF- β 3–STAT6 pathway in chronic airway remodeling with a natural allergen (HDM extracts) (28), as well as indicating its clinical relevance. Thus, our studies represent a novel mechanism of airway remodeling by which Lyn renders an essential negative regulation of TGF- β 3 activities (the pathway is illustrated in Supplemental Fig. 4C).

In summary, we demonstrate that Lyn negatively regulates HDM-induced chronic airway remodeling through the downregulation of TGF- β 3/Smad2, STAT6, and NF- κ B pathways. TGF- β 3 neutralizing Abs inhibited the expression of STAT6, NF- κ B, and Smad2/3, while decreasing phosphorylation of Smad2 and NF- κ B in Lyn^{-/-} mice. AMs and epithelial cells showed increased TGF- β 3 expression upon Lyn knockdown, and Lyn may interact with TGF- β 3 through SH2/SH3 domains. Recombinant TGF- β 3 peptide or adenoviral vector significantly promoted EMT and increased fibroblast collagen I expression. In asthma patients, the expression of Lyn decreased, whereas the expression of TGF- β 3 and Smad2/3 increased, indicating the clinical relevance of our findings based on animal and cellular studies. In conclusion, our studies suggest that Lyn critically suppresses HDM-induced airway remodeling by downregulating the key isoform TGF- β 3.

Acknowledgments

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Disclosures

The authors have no financial conflicts of interest.

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