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Corticosteroids prevent myofibroblast accumulation and airway remodeling in mice

Marina Miller, Jae Youn Cho, Kirsti McElwain, Shauna McElwain, Jung Yeon Shim, Michael Manni, Ji Sun Baek, and David H. Broide

Department of Medicine, University of California San Diego, San Diego, California

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Miller, Marina, Jae Youn Cho, Kirsti McElwain, Shauna McElwain, Jung Yeon Shim, Michael Manni, Ji Sun Baek, and David H. Broide. Corticosteroids prevent myofibroblast accumulation and airway remodeling in mice. *Am J Physiol Lung Cell Mol Physiol* 290: L162–L169, 2006; doi:10.1152/ajplung.00252.2005.—At present there are conflicting results from studies investigating the role of corticosteroids in inhibiting airway remodeling in asthma. We have used a mouse model to determine whether administration of corticosteroids prevents the development of allergen-induced structural features of airway remodeling. Mice treated with corticosteroids were subjected to repetitive ovalbumin (OVA) challenge for 3 mo, at which time levels of peribronchial fibrosis and the thickness of the peribronchial smooth muscle layer were assessed by immunohistology, levels of transforming growth factor (TGF)- β 1 by ELISA, and the number of α -smooth muscle actin+/Col-1+ peribronchial myofibroblasts by immunohistochemistry. Corticosteroids significantly reduced allergen-induced increases in peribronchial collagen deposition and levels of total lung collagen but did not reduce allergen-induced increases in the thickness of the peribronchial smooth muscle layer. Levels of lung TGF- β 1 were significantly reduced in mice treated with systemic corticosteroids, and this was associated with a significant decrease in the number of peribronchial inflammatory cells that expressed TGF- β 1, including eosinophils and mononuclear cells. Corticosteroids also significantly reduced the number of peribronchial myofibroblasts. Overall, these studies demonstrate that administration of corticosteroids significantly reduces levels of allergen-induced peribronchial fibrosis. The reduction in peribronchial fibrosis mediated by corticosteroids is likely to be due to several mechanisms including inhibition of expression of TGF- β 1, a reduction in the number of peribronchial inflammatory cells expressing TGF- β 1 (eosinophils, macrophages), as well as by corticosteroids reducing the accumulation of peribronchial myofibroblasts that contribute to collagen expression.

eosinophil; allergy; transforming growth factor- β 1

CORTICOSTEROIDS ARE CURRENTLY the most effective anti-inflammatory therapy in asthma. They reduce asthma symptoms (16) and frequency of rescue β_2 -adrenergic agonist inhaler use (16), as well as prevent asthma exacerbations (25) and deaths from asthma (30). Corticosteroids are also effective in improving pulmonary function (16) and inhibiting the late-phase lower airway response to allergen challenge (4), as well as reducing airway hyperresponsiveness (16). Several studies using bronchial biopsies have also demonstrated that corticosteroids reduce airway inflammation in asthma (13). Although the ability of corticosteroids to improve these important asthma clinical outcomes and features of airway inflammation is well established, the ability of corticosteroids to reduce airway remodeling is not as well established. There are studies that support

as well as refute a role for corticosteroids in reducing airway remodeling (5, 7, 18, 21, 23, 24, 29, 31, 34). Although the majority of studies demonstrate that corticosteroids reduce features of airway remodeling in asthma (18, 23, 24, 29, 31, 34), several studies do not support a role for corticosteroids in reducing airway remodeling (5, 7, 21). The differences in the results may be due to differences in the dose, duration, and compliance with corticosteroid therapy, as well as the severity of asthma and remodeling end points studied. In addition to these study design issues, bronchial biopsies are taken from large proximal airways in asthma that might not reflect the site of disease activity and airway remodeling in small airways in asthma (20). Postmortem studies of central cartilaginous airways and peripheral membranous small airways in the same asthmatic subject suggest that reticular basement membrane thickness measured in a bronchial biopsy from the central airways reflects airway remodeling in central, but not peripheral, airways (20). Bronchial biopsies also frequently do not contain smooth muscle cells, which are another key component of airway remodeling (22).

We have recently developed a mouse model of airway remodeling in response to repetitive ovalbumin (OVA) challenge that is characterized by structural changes including subepithelial collagen deposition, thickening of the smooth muscle layer, and accumulation of myofibroblasts (8–10, 19). The mouse model of allergen-induced airway remodeling allows us to control many of the variables that are not possible to control in human studies of asthma because of ethical concerns, as well as to optimize the dose and duration of corticosteroid therapy. Studying lung sections from these mice allows the investigation of corticosteroid effects on full-thickness small airways (including smooth muscle), which is not possible in human biopsies of large proximal airways. Thus, in this study, we have investigated whether administration of corticosteroids prevents the development of structural features of airway remodeling in mice exposed to repetitive allergen challenge, as well as determined whether corticosteroids modulate recruitment of myofibroblasts and expression of transforming growth factor (TGF)- β , which can contribute to structural features of airway remodeling.

MATERIALS AND METHODS

Mouse Model of OVA-Induced Airway Remodeling

Eight- to ten-week-old BALB/c mice (16 mice/group; The Jackson Laboratory, Bar Harbor, ME) were immunized sc on days 0, 7, 14, and 21 with 25 μ g of OVA (grade V, Sigma) adsorbed to 1 mg of alum

Address for reprint requests and other correspondence: D. Broide, Univ. of California San Diego, Basic Science Bldg., Rm. 5090, 9500 Gilman Dr., La Jolla, CA 92093-0635 (e-mail: dbroide@ucsd.edu).

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(Aldrich) in 200 μ l of normal saline as previously described (8–10). Intranasal OVA challenges (20 ng/50 μ l in PBS) were administered on days 27, 29, and 31 under isoflurane (Vedco, St. Joseph, MO) anesthesia. Intranasal OVA challenges were then repeated twice a week for 3 mo. Age- and sex-matched control mice were sensitized but not challenged with OVA during the 3-mo study. Mice were killed 24 h after the final OVA challenge, and bronchoalveolar lavage (BAL) fluid and lungs were analyzed. Lungs from the different experimental groups were processed as a batch for either histological staining or immunostaining under identical conditions. Stained and immunostained slides were all quantified under identical light microscope conditions, including magnification ($\times 20$), gain, camera position, and background illumination. The quantitative histological and image analysis of all coded slides was performed by research associates blinded to the coding of all the slides. All animal experimental protocols were approved by the University of California, San Diego Animal Subjects Committee.

Therapeutic Intervention With Corticosteroids

To determine whether corticosteroids could prevent the development of allergen-induced airway remodeling, different groups of mice (16 mice/group) were administered either dexamethasone (1 mg/kg in 100 μ l of sterile, endotoxin-free PBS) or diluent control ip (Fig. 1). The first dose of corticosteroid or diluent was administered 6 h before the first intranasal OVA challenge, and the therapeutic intervention was continued daily for the duration of the 3-mo period of twice weekly OVA challenges. As human studies in asthma have demonstrated differing results based on the dose and duration of corticosteroid therapy (5, 7, 18, 21, 23, 24, 29, 31, 34), in this study systemic as opposed to inhaled corticosteroids were used to determine the maximal effect likely to be mediated by a corticosteroid intervention. The dose of corticosteroid chosen to be used in this study is based on

doses used in previous studies in our laboratory demonstrating that this dose of corticosteroid inhibits allergen-induced eosinophilic inflammation and airway responsiveness in mice. The remodeling end points chosen to test the effect of the corticosteroid intervention included structural changes such as peribronchial fibrosis and thickness of the smooth muscle layer.

Effect of Corticosteroids on the Development of Peribronchial Fibrosis

Peribronchial trichrome staining. Lungs in the different groups of mice were equivalently inflated with an intratracheal injection of a similar volume of 4% paraformaldehyde solution (Sigma Chemicals, St. Louis, MO) to preserve the pulmonary architecture. The area of peribronchial trichrome staining in paraffin-embedded lung was outlined and quantified under a light microscope (Leica DMLS, Leica Microsystems) attached to an image analysis system (Image-Pro plus, Media Cybernetics) as previously described (8–10). Results are expressed as the area of trichrome staining per micrometer length of basement membrane of bronchioles 150–200 μ m of internal diameter.

Lung collagen assay. The amount of lung collagen was measured as previously described in this laboratory (8–10) with a collagen assay kit that uses a dye reagent that selectively binds to the [Gly-X-Y]_n tripeptide sequence of mammalian collagens (Biocolor, Newtownabbey, UK). In all experiments a collagen standard was used to calibrate the assay.

Effect of Corticosteroids on Smooth Muscle Layer and Myofibroblasts

The thickness of the airway smooth muscle layer was measured with an image analysis system as previously described (8–10). In brief, the thickness of the smooth muscle layer (the transverse diameter) was measured from the innermost aspect to the outermost aspect

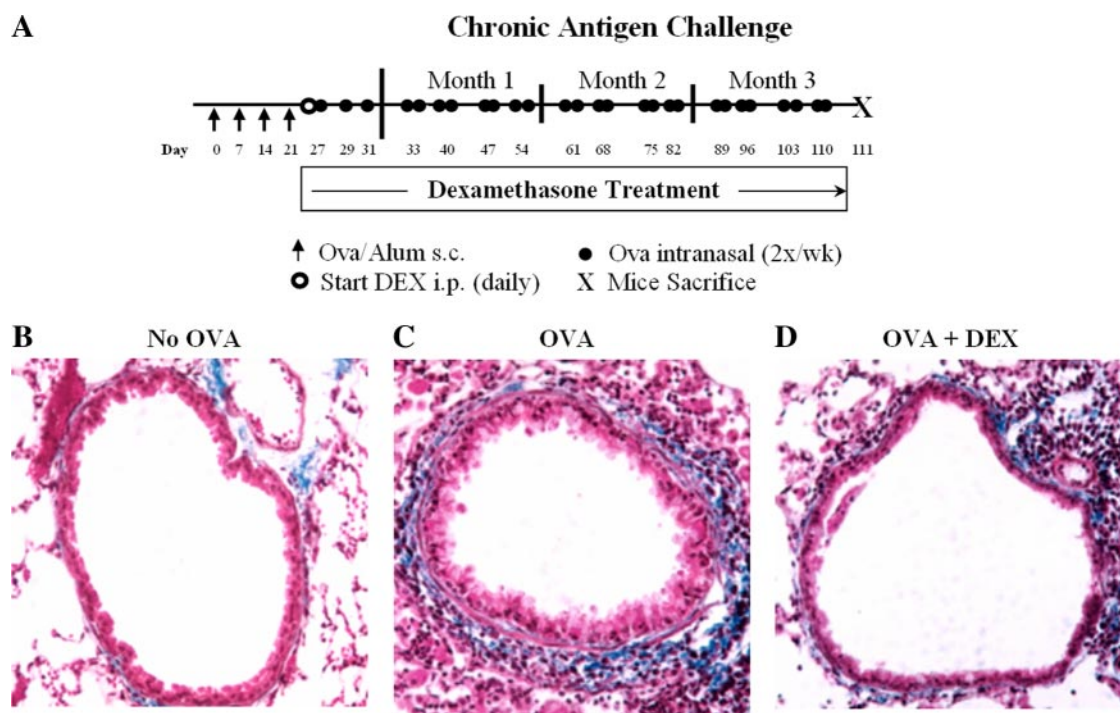


Fig. 1. Mouse ovalbumin (OVA) experimental protocol. A: outline of the experimental protocol. Mice were immunized sc on days 0, 7, 14, and 21 with OVA (arrows). Intranasal OVA challenges were administered on days 27, 29, and 31 and then repeated twice a week for 3 mo (●). Age- and sex-matched control mice were sensitized but not challenged with OVA during the 3-mo study. Mice were killed 24 h after the final OVA challenge, and bronchoalveolar lavage (BAL) fluid and lungs were analyzed. Corticosteroids [dexamethasone (Dex)] or diluent control was administered ip starting on day 26, 1 day before the first intranasal OVA challenge (○), and then continued daily for 3 mo. Trichrome-stained (blue color) lung sections of either non-OVA-challenged control mice (B), mice challenged with OVA for 3 mo (C), or mice challenged with OVA for 3 mo and treated with Dex (D).

of the smooth muscle layer. The smooth muscle layer thickness in at least 10 bronchioles of similar size (150–200 μm) was counted on each slide. Lung sections were also immunostained with both an anti- α -smooth muscle actin primary antibody (Sigma-Aldrich) and an anti-Col-1 primary antibody (Chemicon) to distinguish myofibroblasts (α -smooth muscle actin positive; Col-1 positive) (17, 35) that express collagen (Col-1) from smooth muscle cells (α -smooth muscle actin positive; Col-1 negative). Species- and isotype-matched antibodies were used as controls in place of the primary antibodies. The primary antibodies were detected with two different horseradish peroxidase (HRP) enzyme-labeled secondary antibodies with signal amplification using tyramide signal amplification (Molecular Probes) according to the manufacturer's instructions. The anti- α -smooth muscle actin primary antibody was detected with an HRP-labeled secondary antibody (Alexa 546, red color), while the anti-Col-1 primary antibody was detected with a different HRP-labeled secondary antibody (Alexa 488, green color). Cells coexpressing α -smooth muscle actin and Col-1 have a yellow color.

Effect of Corticosteroids on TGF- β 1 Expression

The concentrations of TGF- β 1 in lung were assayed by ELISA (R&D Systems) as previously described in this laboratory's study (8–10). The number of peribronchial cells expressing TGF- β 1 were assessed in lung sections processed for TGF- β 1 immunohistochemistry as previously described (8–10).

Effects of Corticosteroids on Fibronectin Expression

Levels of peribronchial fibronectin expression were assessed in lung sections immunostained with a polyclonal antifibronectin antibody (Sigma) that recognizes both cellular and plasma fibronectin. The area of peribronchial fibronectin immunostaining was outlined and quantified under a light microscope attached to an image analysis system as described in *Peribronchial trichrome staining*. Results are expressed as the area of fibronectin staining per micrometer length of basement membrane of bronchioles 150–200 μm of internal diameter.

Effect of Corticosteroids on Airway Mucus Expression and Inflammation

The number of periodic acid-Schiff (PAS)-positive and PAS-negative epithelial cells in individual bronchioles were counted as previously described in this laboratory (8–10). BAL total eosinophil counts and immunohistochemical detection of peribronchial CD4+ T lymphocytes and F4/80-positive mononuclear cells were assessed as previously described (8–10).

Statistical Analysis

Results in the different groups of mice were compared by ANOVA using the nonparametric Kruskal-Wallis test followed by posttesting using Dunn's multiple comparison of means. All results are presented as means $\pm$ SE. A statistical software package (Graph Pad Prism, San Diego, CA) was used for the analysis. *P* values of <0.05 were considered statistically significant.

RESULTS

Corticosteroids Reduce OVA-Induced Peribronchial Collagen Deposition

Lung collagen. Mice challenged repetitively with OVA for 3 mo had a significant increase in the levels of lung collagen compared with non-OVA-challenged mice ($1,286 \pm 106$ vs. 653 ± 152 μg collagen/lung, OVA vs. no-OVA; *P* = 0.01; Fig. 2A). To determine whether corticosteroids could reduce OVA-induced increases in lung collagen deposition, dexamethasone was administered daily for 3 mo starting before the

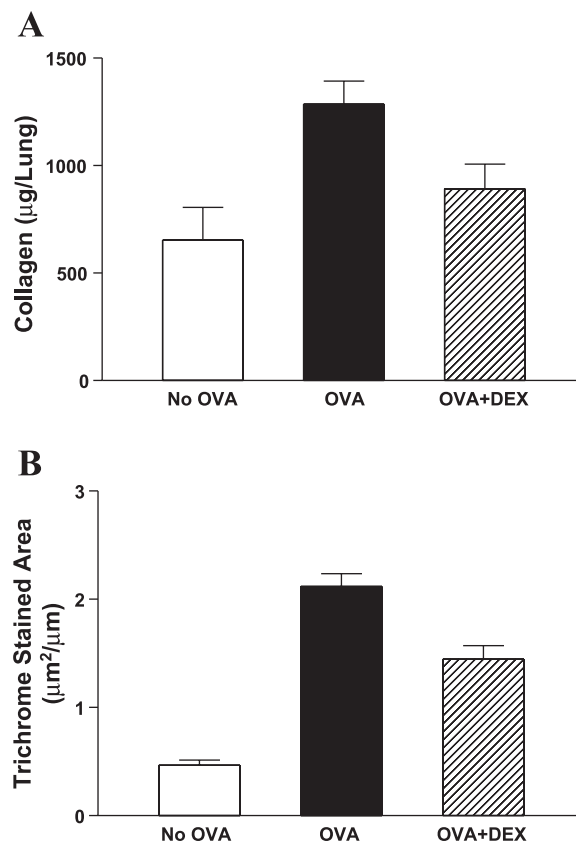


Fig. 2. Corticosteroids reduce peribronchial fibrosis in OVA-challenged mice. **A:** effect of Dex on levels of total lung collagen. Mice repetitively challenged with OVA for 3 mo had a significant increase in levels of lung collagen compared with control non-OVA-challenged mice (OVA vs. no-OVA, *P* = 0.01). Administration of Dex ip for 3 mo to mice repetitively challenged with OVA significantly reduced levels of lung collagen (OVA vs. OVA + Dex, *P* = 0.05, *n* = 16 mice/group). **B:** effect of Dex on levels of peribronchial trichrome staining. Mice repetitively challenged with OVA for 3 mo had a significant increase in the area of peribronchial trichrome staining compared with control non-OVA-challenged mice (OVA vs. no-OVA, *P* = 0.001). Administration of Dex ip for 3 mo to mice repetitively challenged with OVA significantly reduced levels of peribronchial trichrome staining (OVA vs. OVA + Dex, *P* = 0.01, *n* = 16 mice/group).

first intranasal OVA challenge. Dexamethasone significantly prevented lung collagen deposition in mice subjected to repetitive OVA challenge for 3 mo (892 ± 115 vs. $1,286 \pm 106$ μg collagen/lung, Dex + OVA vs. OVA; *P* = 0.05; Fig. 2A).

Peribronchial trichrome stain. Repetitive OVA challenge for 3 mo in untreated mice induced a significant increase in the area of peribronchial trichrome staining compared with non-OVA challenged mice (2.12 ± 0.12 vs. 0.47 ± 0.05 $\mu\text{m}^2/\mu\text{m}$ circumference of bronchiole, OVA vs. no-OVA; *P* = 0.001; Figs. 1, B and C, and 2B). Dexamethasone significantly reduced the area of peribronchial trichrome staining in mice subjected to repetitive OVA challenge (1.44 ± 0.12 vs. 2.12 ± 0.12 $\mu\text{m}^2/\mu\text{m}$ circumference of bronchiole, Dex + OVA vs. OVA; *P* = 0.01; Figs. 1, C and D, and 2B).

Corticosteroids Reduce the Area of Peribronchial α -Smooth Muscle Actin Immunostaining and the Number of Myofibroblasts

Repetitive OVA challenge for 3 mo induced a significant increase in the area of peribronchial α -smooth muscle actin



immunostaining (0.86 ± 0.04 vs. $0.35 \pm 0.03 \mu\text{m}^2/\mu\text{m}$, OVA vs. no-OVA; $P = 0.001$; Fig. 3A) as well as a significant increase in the thickness of the smooth muscle layer (4.66 ± 0.19 vs. $2.91 \pm 0.12 \mu\text{m}$, OVA vs. no-OVA; $P = 0.001$; Fig. 3B). Dexamethasone significantly reduced the area of peribronchial α -smooth muscle actin staining in mice subjected to repetitive OVA challenge (0.58 ± 0.03 vs. $0.86 \pm 0.04 \mu\text{m}^2/\mu\text{m}$ circumference of bronchiole, Dex + OVA vs. OVA; $P = 0.001$; Fig. 3A). Although dexamethasone significantly reduced the area of peribronchial α -smooth muscle actin staining in mice subjected to repetitive OVA challenge, dexamethasone did not similarly reduce the thickness of the peribronchial smooth muscle layer in mice subjected to repetitive OVA challenge (4.32 ± 0.12 vs. $4.66 \pm 0.19 \mu\text{m}$, Dex + OVA vs. OVA; $P =$ not significant; Fig. 3B). This discrepancy suggested that dexamethasone might not be reducing the number of peribronchial α -smooth muscle actin-positive cells that were smooth muscle but could be reducing levels of peribronchial α -smooth muscle actin-positive cells that were myofibroblasts (which express α -smooth muscle actin and Col-1) (17, 35). We therefore performed double-label immunohistochemistry ex-

periments to detect peribronchial cells expressing both α -smooth muscle actin and Col-1 (Fig. 3, C–E). Repetitive OVA challenge for 3 mo induced a significant increase in α -smooth muscle actin-positive/Col-1-positive peribronchial cells compared with non-OVA-challenged mice (33.2 ± 2.4 vs. $14.7 \pm 1.4 \mu\text{m}^2/\mu\text{m}$, OVA vs. no-OVA; $P = 0.001$; Fig. 3F). Dexamethasone significantly reduced the number of α -smooth muscle actin-positive/Col-1-positive peribronchial cells in mice subjected to repetitive OVA challenge (24.2 ± 2.0 vs. $33.2 \pm 2.4 \mu\text{m}^2/\mu\text{m}$ circumference of bronchiole, Dex + OVA vs. OVA; $P = 0.05$; Fig. 3F).

Effect of Corticosteroids on TGF- β 1 Expression

Lung TGF- β 1. Repetitive OVA challenge induced a significant increase in levels of lung TGF- β 1 ($3,312 \pm 715$ vs. 938 ± 204 pg/ml, OVA vs. non-OVA; $P = 0.05$; Fig. 4A). Dexamethasone significantly reduced levels of lung TGF- β 1 in mice subjected to repetitive OVA challenge ($1,270 \pm 185$ vs. $3,312 \pm 715$ pg/ml, Dex + OVA vs. OVA; $P = 0.05$; Fig. 4A).

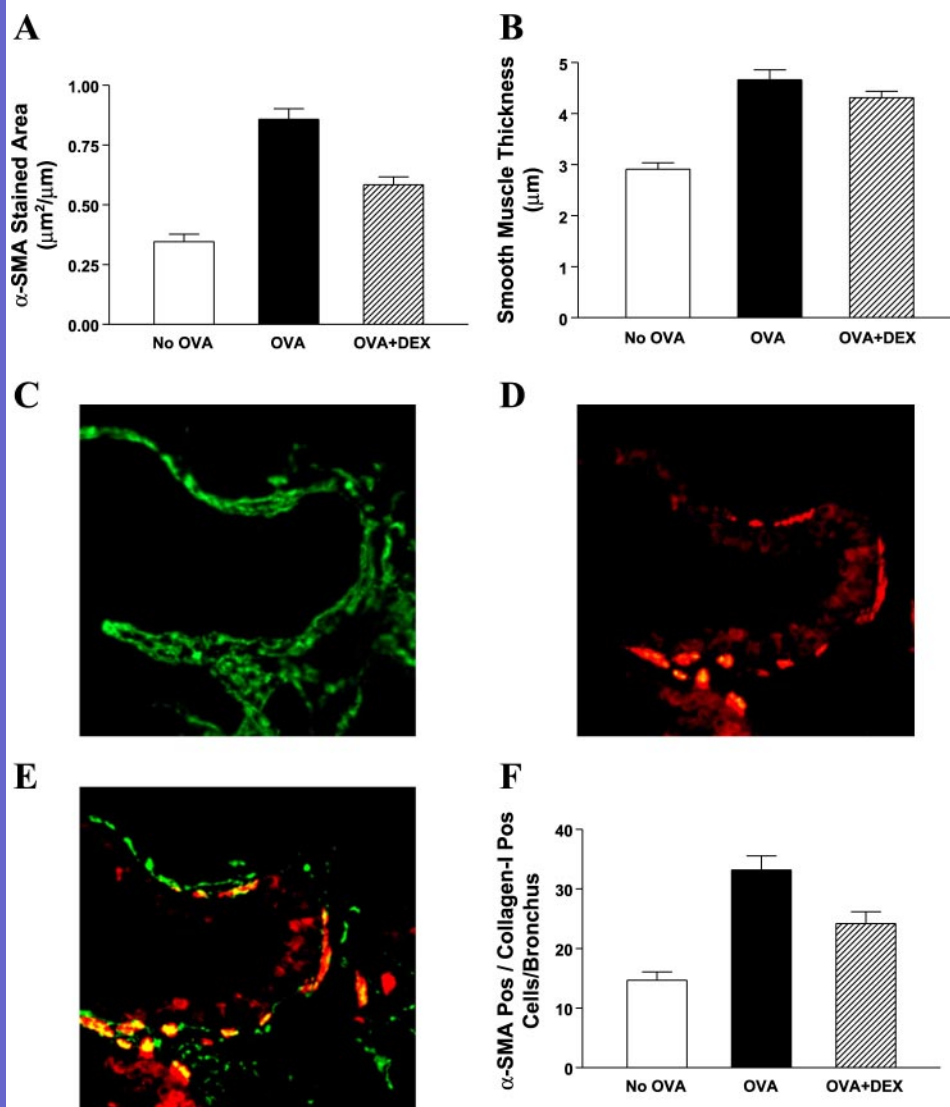


Fig. 3. Corticosteroids reduce peribronchial myofibroblast accumulation. **A:** effect of corticosteroids on levels of peribronchial α -smooth muscle actin (SMA) immunostaining. Mice repetitively challenged with OVA for 3 mo had a significant increase in levels of peribronchial α -SMA immunostaining compared with control non-OVA-challenged mice (OVA vs. no-OVA, $P = 0.001$). Administration of Dex ip for 3 mo to mice repetitively challenged with OVA significantly reduced levels of peribronchial α -SMA immunostaining (OVA vs. OVA + Dex, $P = 0.001$, $n = 16$ mice/group). **B:** effect of corticosteroids on smooth muscle thickness. Mice repetitively challenged with OVA for 3 mo had a significant increase in levels of peribronchial smooth muscle thickness compared with control non-OVA-challenged mice (OVA vs. no-OVA, $P = 0.001$). Administration of Dex ip for 3 mo to mice repetitively challenged with OVA did not significantly reduce levels of peribronchial smooth muscle thickness (OVA vs. OVA + Dex, $P =$ ns, $n = 16$ mice/group). **C–E:** detection of peribronchial cells coexpressing α -smooth muscle actin and Col-1. Mice repetitively challenged with OVA for 3 mo had a significant increase in the number of peribronchial cells expressing Col-1 (immunofluoresce green in C) as well as cells expressing α -SMA (immunofluoresce red in D). Peribronchial cells expressing both α -SMA and Col-1 immunofluoresce yellow (E). **F:** effect of corticosteroids on the number of peribronchial cells coexpressing α -SMA and Col-1. Mice repetitively challenged with OVA for 3 mo had a significant increase in levels of peribronchial cells expressing both α -SMA and Col-1 compared with control non-OVA-challenged mice (OVA vs. no-OVA, $P = 0.001$). Administration of Dex ip for 3 mo to mice repetitively challenged with OVA significantly reduced levels of peribronchial cells expressing both α -SMA and Col-1 (OVA vs. OVA + Dex, $P = 0.05$, $n = 16$ mice/group).

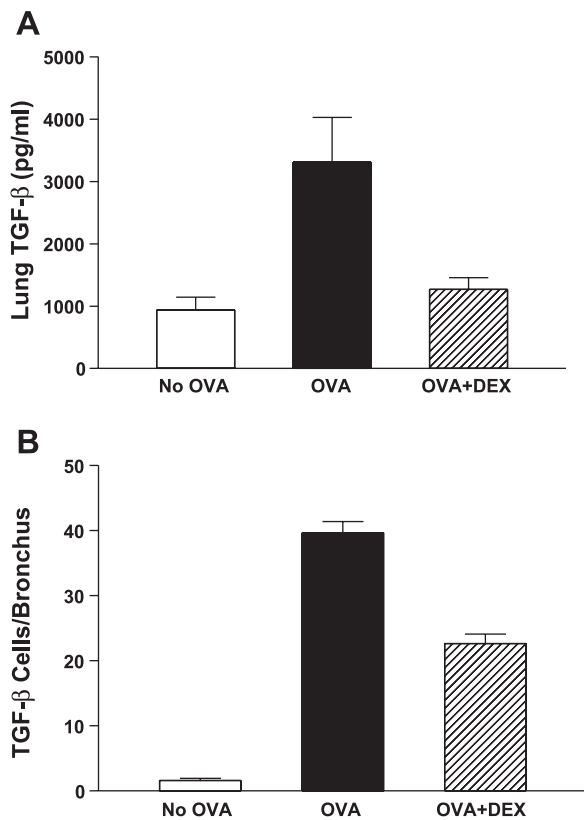


Fig. 4. Corticosteroids reduce levels of transforming growth factor (TGF)- β 1. A: lung TGF- β 1. Mice repetitively challenged with OVA for 3 mo had increased levels of lung TGF- β 1 compared with control non-OVA-challenged mice (OVA vs. no OVA, $P = 0.05$). Administration of Dex ip for 3 mo to mice repetitively challenged with OVA significantly reduced levels of lung TGF- β 1 (OVA vs. OVA + Dex, $P = 0.05$, $n = 16$ mice/group). B: peribronchial TGF- β 1-positive cells. Mice repetitively challenged with OVA for 3 mo had a significant increase in the number of peribronchial cells immunostaining positive for TGF- β 1 compared with control non-OVA-challenged mice (OVA vs. no OVA, $P = 0.001$). Administration of Dex ip for 3 mo to mice repetitively challenged with OVA significantly reduced the number of peribronchial cells immunostaining positive for TGF- β 1 (OVA vs. OVA + Dex, $P = 0.01$, $n = 16$ mice/group).

Peribronchial TGF- β 1-positive cells. Repetitive OVA challenge induced a significant increase in the number of peribronchial TGF- β 1-positive cells (39.7 ± 1.7 vs. 1.6 ± 0.3 TGF- β -positive cells/bronchus, OVA vs. non-OVA; $P = 0.001$; Fig. 4B). Dexamethasone significantly reduced the number of peribronchial TGF- β 1-positive cells in mice subjected to repetitive OVA challenge (22.6 ± 1.5 vs. 39.7 ± 1.7 TGF- β -positive cells/bronchus, Dex + OVA vs. OVA; $P = 0.01$; Fig. 4B).

Effect of Corticosteroids on Levels of Fibronectin Expression

OVA challenge induced significant increased levels of fibronectin expression (OVA vs. non-OVA, $P = 0.001$, Fig. 5). Dexamethasone significantly reduced the area of peribronchial fibronectin staining in mice subjected to repetitive OVA challenge (3.84 ± 0.23 vs. $1.51 \pm 0.18 \mu\text{m}^2/\mu\text{m}$ circumference of bronchiole, Dex + OVA vs. OVA; $P = 0.001$; Fig. 5).

Effect of Corticosteroids on Airway Mucus Expression and Inflammation

Repetitive OVA challenge for 3 mo in untreated mice induced a significant increase in the percentage of airway

epithelium that stained positive with PAS compared with non-OVA-challenged mice (50.8 ± 2.7 vs. $0.3 \pm 0.01\%$ PAS-positive cells/bronchus, OVA vs. non-OVA; $P = 0.001$; Fig. 6A). Although dexamethasone significantly reduced the percentage of PAS-positive stained cells mice in mice subjected to repetitive OVA challenge (40.1 ± 2.7 vs. $50.8 \pm 2.7\%$ PAS-positive cells/bronchus, Dex + OVA vs. OVA; $P = 0.05$; Fig. 6A), there was still considerable mucus expression in dexamethasone-treated mice.

Repetitive OVA challenge for 3 mo in untreated mice induced a significant increase in the number of BAL eosinophils ($P = 0.001$, Fig. 6B), peribronchial CD4+ cells ($P = 0.001$, Fig. 6C), and peribronchial F4/80+ mononuclear cells ($P = 0.001$, Fig. 6D). Dexamethasone therapy in OVA-challenged mice significantly reduced levels of BAL eosinophils ($P = 0.05$, Fig. 6B), peribronchial CD4+ cells ($P = 0.001$, Fig. 6C), and peribronchial F4/80+ mononuclear cells ($P = 0.001$, Fig. 6D).

DISCUSSION

In this study we have demonstrated that corticosteroids significantly reduce peribronchial fibrosis (assessed by total lung collagen and peribronchial trichrome staining) induced by repetitive allergen challenge in a mouse model of airway remodeling. We have also demonstrated potential mechanism(s) by which corticosteroids may reduce allergen-induced peribronchial collagen deposition through inhibition of expression of the profibrotic growth factor TGF- β 1 as well as inhibition of accumulation of myofibroblasts. Although this study has focused on the ability of corticosteroids to inhibit airway remodeling through effects on TGF- β 1 and myofibroblasts, corticosteroids may also potentially inhibit airway remodeling through effects on pathways that have not been investigated in this study including inhibition of expression of profibrotic cytokines other than TGF- β 1, as well as direct inhibitory effects on fibroproliferation, the extracellular matrix, and edema. As the extent of peribronchial fibrosis is a balance between the synthesis and breakdown of collagen, effects of corticosteroids that either decrease synthesis and/or increase breakdown of collagen will likely reduce allergen-induced peribronchial fibrosis. Many lung cells can produce TGF- β 1,

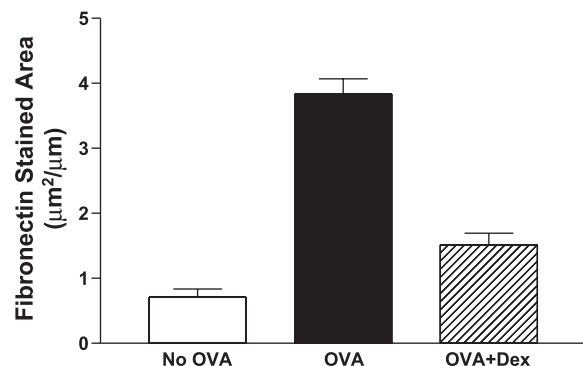


Fig. 5. Effect of corticosteroids on levels of peribronchial fibronectin immunostaining. Mice repetitively challenged with OVA for 3 mo had a significant increase in levels of peribronchial fibronectin immunostaining compared with control non-OVA challenged mice (OVA vs. no-OVA, $P = 0.001$). Administration of Dex ip for 3 mo to mice repetitively challenged with OVA significantly reduced levels of peribronchial fibronectin immunostaining (OVA vs. OVA + Dex, $P = 0.001$, $n = 16$ mice/group).

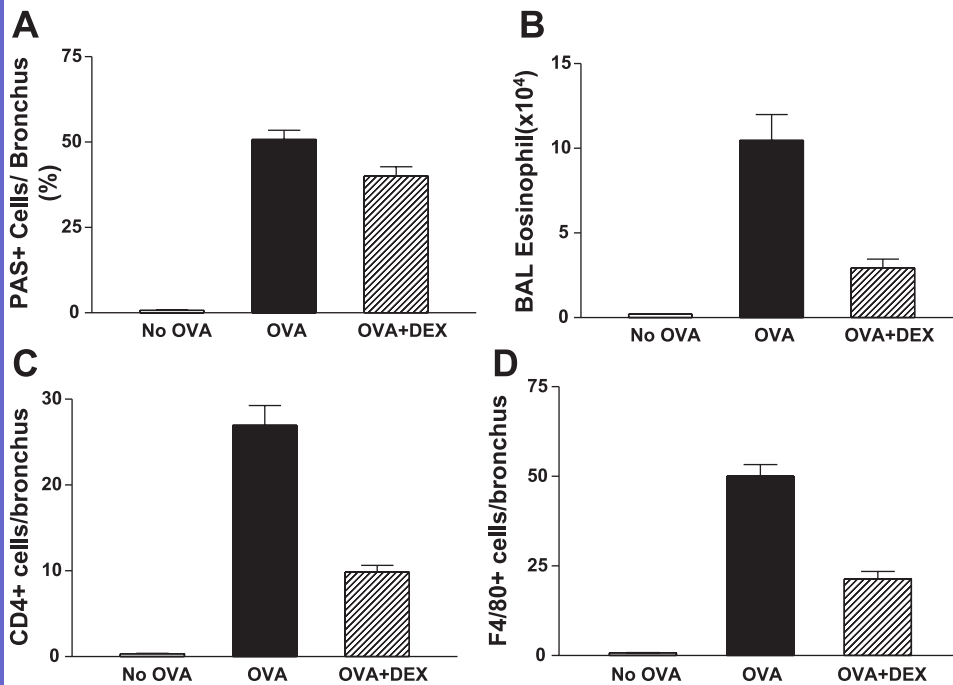


Fig. 6. Corticosteroids reduce airway mucus expression and inflammation. Mice repetitively challenged with OVA for 3 mo had a significant increase in the number of airway epithelial cells staining positive for periodic acid-Schiff (PAS) (OVA vs. no OVA, $P = 0.001$; A), as well as BAL eosinophils ($P = 0.001$, B), CD4+ cells ($P = 0.001$, C), and F4/80+ cells (D, $P = 0.001$). Administration of Dex ip for 3 mo to mice repetitively challenged with OVA significantly reduced the number of airway epithelial cells staining positive for PAS (OVA vs. OVA + Dex, $P = 0.05$; A), as well as reduced the number of BAL eosinophils ($P = 0.05$, B), CD4+ cells ($P = 0.001$, C), and F4/80+ cells (D; $P = 0.001$, $n = 16$ mice/group).

but the important sources in the airway include eosinophils, macrophages, and epithelium (2, 9, 14). Our studies suggest that corticosteroids reduce levels of lung TGF- β 1 and reduce the number of peribronchial inflammatory cells that express TGF- β 1 (mononuclear cells and eosinophils). TGF- β 1 is a potent profibrotic cytokine that stimulates fibroblasts to promote the synthesis and secretion of many extracellular matrix proteins including collagen I, collagen III, fibronectin, vitronectin, tenascin, as well as proteoglycans (2). TGF- β 1 also decreases the synthesis of enzymes that degrade the extracellular matrix, such as matrix metalloproteinases, and increases the synthesis of inhibitors of these enzymes, such as tissue inhibitor of metalloproteinase-1 (2). TGF- β 1 expression correlates with the degree of subepithelial fibrosis, and levels of TGF- β 1 are significantly increased in patients with severe asthma who have prominent airway eosinophilic inflammation (26, 33). Thus the ability of corticosteroids to inhibit allergen-induced TGF- β 1 expression could play a significant role in how corticosteroids inhibit airway remodeling in asthma.

In addition to its effects on fibrosis, TGF- β 1 is also important in inducing differentiation of fibroblasts to myofibroblasts (12). Myofibroblasts are specialized cells with features of both fibroblasts and myocytes. They have the synthetic machinery of fibroblasts that is used for the synthesis of the extracellular matrix and also have some of the components of the contractile apparatus of myocytes. We have demonstrated that corticosteroids reduce both levels of TGF- β 1 as well as the numbers of peribronchial myofibroblasts, suggesting that corticosteroid-mediated inhibition of TGF- β 1 could result in reduced differentiation of fibroblasts into myofibroblasts. However, it is also possible that corticosteroids may have reduced the numbers of myofibroblasts through effects on myofibroblast proliferation and/or induced myofibroblast apoptosis. Interestingly, specific isoforms of fibronectin appear to be necessary for the induction of the myofibroblast phenotype by TGF- β 1 (28). If corticosteroids reduce expression of the fibronectin isoform containing

the Extra-Domain-A (ED-A) in the remodeled airway, this could contribute to reduced TGF- β 1-induced differentiation of myofibroblasts (28). In this study we demonstrated that corticosteroids reduced peribronchial fibronectin expression. Further studies are needed to determine whether corticosteroids reduce levels of individual isoforms of fibronectin. As myofibroblasts are important contributors to production of collagen and extracellular matrix proteins in the remodeled airway in asthma (6, 15, 27), corticosteroid-induced reductions in the number of peribronchial myofibroblasts may play an important role in reducing airway remodeling. In humans with mild asthma, allergen challenge induces an increase in levels of BAL TGF- β (1), as well as an accumulation of myofibroblasts in mucosal biopsies 24 h after allergen challenge (15). Thus the ability of corticosteroids to inhibit expression of TGF- β and inhibit accumulation of myofibroblasts may be important mechanisms by which corticosteroids mediate their inhibitory effect on the development of allergen-induced peribronchial fibrosis. Interestingly, studies in mice (9) and humans (14) have shown an important role for TGF- β in airway remodeling as depletion of eosinophils expressing TGF- β with anti-IL-5 leads to a reduction in both eosinophils as well as a reduction in levels of lung TGF- β and airway remodeling.

Studies have also examined the role of corticosteroids in mouse (3, 11) and rat (32) models of asthma. In previous studies examining airway remodeling in a mouse model of asthma, three intratracheal instillations of OVA antigen over a 10-day period to mice sensitized to OVA induced a 1.4-fold increase in subepithelial reticulin, which was reduced by dexamethasone treatment (3). Recent studies in mice have also demonstrated that dexamethasone reduces levels of laminin and laminin-1 receptor expression (11). In rats, OVA challenges for 2 wk increase levels of the extracellular matrix protein fibronectin, as assessed by immunohistochemistry, and airway wall thickness (32). As rats exposed to OVA challenge do not develop thickening of the smooth muscle layer (32),



studies related to corticosteroid effects on this smooth muscle remodeling end point are not possible in this rat model but are possible in the mouse model that we have developed. The studies in rats have demonstrated that corticosteroids reduce levels of fibronectin and airway wall thickness (32). In this study in mice we have demonstrated not only by collagen staining methods, but also by measuring total lung collagen that corticosteroids reduce levels of allergen-induced peribronchial collagen deposition. In addition, we have described a potential mechanism by which corticosteroids may be reducing peribronchial fibrosis in vivo by reducing levels of TGF- β 1, the number of TGF- β 1-positive peribronchial cells, and the accumulation of peribronchial myofibroblasts.

In summary, this study demonstrates that administration of corticosteroids can prevent allergen-induced increases in peribronchial fibrosis. The ability of corticosteroids to inhibit allergen-induced peribronchial fibrosis is likely mediated by several mechanisms, including inhibition of TGF- β 1 expression, reductions in the number of peribronchial cells that express TGF- β 1, and reductions in peribronchial myofibroblast accumulation. These observations with corticosteroids in a mouse model of airway remodeling suggest a potential mechanism, through effects on TGF- β 1 expression and myofibroblast accumulation, to account for the ability of corticosteroids to reduce features of airway remodeling in asthmatics (18, 23, 24, 29, 31, 34). However, corticosteroids may also have additional effects on the synthesis or degradation of collagen that could account for reductions in peribronchial fibrosis. Future studies in human asthmatics using inhibitors of TGF- β will help to determine whether or not inhibition of TGF- β is a key mechanism by which corticosteroids inhibit airway remodeling in asthma.

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