

Allergen-induced peribronchial fibrosis and mucus production mediated by I κ B kinase β -dependent genes in airway epithelium

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Contributed by Michael Karin, October 24, 2005

In response to inflammation or injury, airway epithelial cells express inducible genes that may contribute to allergen-induced airway remodeling. To determine the contribution of epithelial cell NF- κ B activation to the remodeling response, we generated *CC10-Cre^{tg}/Ikk β ^{Δ/Δ}* mice in which NF- κ B signaling through I κ B kinase β (IKK β) is selectively ablated in the airway epithelium by conditional Cre-recombinase expression from the Clara cell (CC10) promoter. Repetitive ovalbumin challenge of mice deficient in airway epithelial IKK β prevented nuclear translocation of the RelA NF- κ B subunit only in airway epithelial cells, resulting in significantly lower peribronchial fibrosis in *CC10-Cre^{tg}/Ikk β ^{Δ/Δ}* mice compared with littermate controls as assessed by peribronchial trichrome staining and total lung collagen content. Levels of airway mucus, airway eosinophils, and peribronchial CD4⁺ cells in ovalbumin-challenged mice were also reduced significantly upon airway epithelial *Ikk β* ablation. The diminished inflammatory response was associated with reduced expression of NF- κ B-regulated chemokines, including eotaxin-1 and thymus- and activation-regulated chemokine, which attract eosinophils and Th2 cells, respectively, into the airway. The number of peribronchial cells expressing TGF- β 1, as well as TGF- β 1 amounts in bronchoalveolar lavage, were also significantly reduced in mice deficient in airway epithelium IKK β . Overall, these studies show an important role for NF- κ B regulated genes in airway epithelium in allergen-induced airway remodeling, including peribronchial fibrosis and mucus production.

asthma | NF- κ B | eosinophil | TGF- β

The airway inflammatory response in asthma is characterized by inducible expression of multiple genes encoding cytokines, chemokines, and adhesion molecules, which are associated with recruitment of eosinophils and Th2 lymphocytes (1). In addition to the characteristic Th2-mediated eosinophilic inflammatory response found in the airway during acute asthma episodes, chronic asthma is characterized by structural changes that are termed airway remodeling (1, 2). Remodeling-associated changes in the airway include peribronchial fibrosis with increased deposition of collagen (types I, III, and V), smooth muscle hypertrophy/hyperplasia, and mucus secretion (1, 2). Repeated cycles of inflammation and repair in the airway in chronic asthma are considered to be the driving force for airway remodeling. Recent studies in mice (3, 4) and humans (5) have supported an important role for eosinophilic inflammation in allergen-induced airway remodeling. In allergen-challenged IL-5-deficient mice, depletion of eosinophils results in significantly reduced levels of eosinophilic airway inflammation, reduced eosinophil TGF- β 1 expression, and less airway remodeling (3). Similarly, in humans with mild chronic asthma, depletion of eosinophils with anti-IL-5 antibody (Ab) reduces levels of airway eosinophils, eosinophil TGF- β 1 expression, and airway remodeling (5).

In addition to inflammatory cells (eosinophils and Th2 cells), structural cells such as epithelial cells also were hypothesized to

contribute to airway remodeling in asthma (6). In asthma the bronchial epithelium is abnormal, with structural changes including separation of columnar cells from their basal membrane attachments and functional changes including increased expression and release of proinflammatory cytokines, growth factors, and mediator-generating enzymes (6). To start understanding whether genes expressed by the airway epithelium contribute to allergen-induced airway remodeling, we used the *Cre/loxP* approach (7) to selectively inactivate in airway epithelium the gene encoding I κ B kinase β (IKK β), which is required for activation of transcription factor NF- κ B (8, 9). Because NF- κ B regulates the epithelial expression of multiple genes that may be important to airway remodeling, including genes encoding cytokines, chemokines, and adhesion molecules (10), this approach should enable us to determine both the role of IKK β and NF- κ B regulated genes in mediating airway remodeling, as well as gain insight into the contribution of the airway epithelium to remodeling response.

Dimeric NF- κ B transcription factors regulate gene transcription by binding to specific κ B elements in the promoter regions of target genes (10, 11). In unstimulated cells, most NF- κ B dimers are retained in the cytoplasm by means of association with specific inhibitors termed I κ Bs (10, 11). Upon cell stimulation, the I κ Bs are phosphorylated and degraded, and liberated NF- κ B dimers enter the nucleus to up-regulate genes containing κ B elements in their regulatory regions. This pathway of NF- κ B activation depends on the IKK complex, whose activity is stimulated by proinflammatory stimuli such as TNF (10, 11), a cytokine expressed in the airway in asthma (12), as well as by viruses and bacteria that activate Toll-like receptors (11). IKK is a multisubunit kinase complex composed of the catalytic subunits IKK α and IKK β and the regulatory subunit IKK γ [also known as NF- κ B essential modulator (NEMO); refs. 10 and 11]. One of the major functions of the IKK complex is to phosphorylate I κ B molecules, thus triggering their degradation and the activation of NF- κ B. Although both IKK α and IKK β can phosphorylate all three I κ B proteins *in vitro*, studies in mice that are deficient in IKK subunits show that, in most cells, IKK β has the dominant role in signal-induced phosphorylation and degradation (10, 11).

Evidence that NF- κ B may have an important role in asthma is derived from both animal models of asthma in which components of NF- κ B have been inactivated (13–15), as well as from human studies showing that airway epithelial cells in bronchial

Conflict of interest statement: No conflicts declared.

Abbreviations: OVA, ovalbumin; IKK β , I κ B kinase β ; rTAT, reverse tetracycline-responsive transactivator; tetO, tet-operator; BAL, bronchoalveolar lavage; TARC, thymus- and activation-regulated chemokine.

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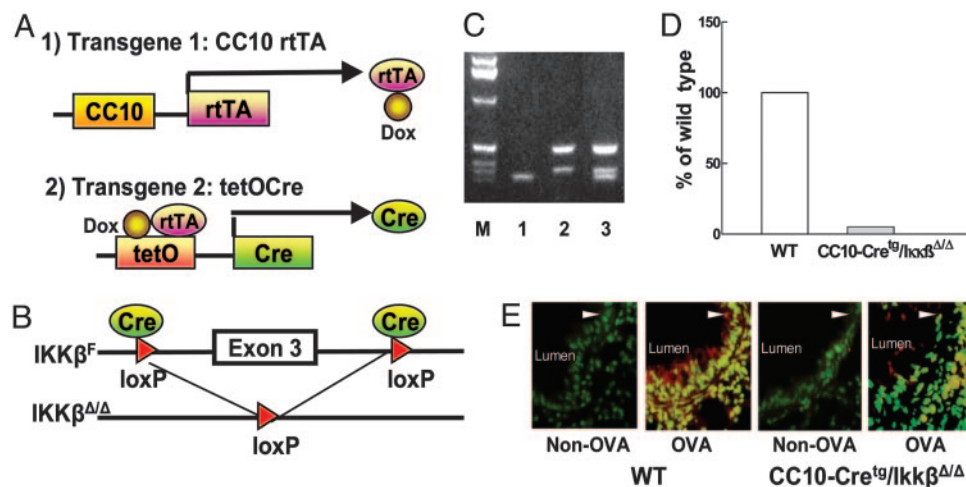


Fig. 1. Generation of mice lacking IKK β in airway epithelium. Genotyping of CC10-Cre^{tg}/Ikk $\beta^{\Delta/\Delta}$ mice. CC10-Cre^{tg} mice (A) were crossed with Ikk $\beta^{F/F}$ mice (B) to generate, after doxycycline administration, CC10-Cre^{tg}/Ikk $\beta^{\Delta/\Delta}$ progeny in which the Ikk β^F allele is selectively deleted in airway epithelium. (A1) In CC10-Cre^{tg} mice the CC10 promoter induces selective airway epithelial expression of rtTA. (A2) In the presence of doxycycline, rtTA binds to a tetO-containing promoter activating transcription of a transgene encoding Cre-recombinase in the airway epithelium. (B) Cre-recombinase cleaves at the loxP sites that flank exon 3 of the Ikk β^F allele to produce the deleted Ikk β^{Δ} allele, which is null for IKK β expression. (C) RT-PCR analysis of CC10-Cre^{tg}/Ikk $\beta^{F/F}$ progeny (lane 3) indicates the presence of both transgenes contributed by the CC10-Cre^{tg} mice (lane 2), as well as the Ikk β^F allele, contributed by the Ikk $\beta^{F/F}$ parents (lane 1). (D) TaqMan PCR studies showing that, in CC10-Cre^{tg}/Ikk $\beta^{\Delta/\Delta}$ mice, sequences specific to Ikk β exon 3 were absent in airway epithelial cells. (E) Activation of NF- κ B after OVA challenge: Immunofluorescence microscopy of bronchiole sections stained with anti-RelA Abs and nuclear counterstaining with SYTOX green. RelA is cytoplasmic (green) in airway epithelial cells (arrow) as well as in peribronchial mononuclear cells of non-OVA-challenged WT and CC10-Cre^{tg}/Ikk $\beta^{\Delta/\Delta}$ mice. OVA-challenged WT mice exhibit nuclear RelA staining (yellow) in both airway epithelial cells (arrow) and peribronchial mononuclear cells. In OVA-challenged CC10-Cre^{tg}/Ikk $\beta^{\Delta/\Delta}$ mice RelA remained cytoplasmic in most airway epithelial cells (arrow) but was nuclear in peribronchial cells, which were reduced in number in CC10-Cre^{tg}/Ikk $\beta^{\Delta/\Delta}$ compared with WT mice.

biopsies have increased nuclear levels of the RelA (p65) NF- κ B subunit compared with specimens from normal subjects (16). Electrophoretic mobility-shift assays and immunohistochemical studies also suggest a greater activation of RelA-containing NF- κ B dimers in severe asthmatics (17). In mouse models of asthma, mice deficient in either the p50 (13, 14) or the c-Rel (15) NF- κ B subunits, or mice treated with inhibitors of epithelial NF- κ B activity (18), have significantly reduced levels of eosinophilic lung inflammation when challenged with inhaled allergen. In this study, we investigated the contribution of epithelial genes regulated by NF- κ B to allergen-induced airway remodeling after chronic ovalbumin (OVA) challenge. Whereas previous studies have addressed the role of NF- κ B in acute airway inflammation, this study provides evidence for a critical role of epithelial NF- κ B in airway remodeling caused by chronic inflammation in asthma.

Methods

Mice. To selectively inactivate NF- κ B signaling in airway epithelial cells, the gene encoding Ikk β was targeted by using the Cre/loxP procedure in Ikk $\beta^{F/F}$ mice (background strain C57/BL), which are homozygous for a “floxed” Ikk β allele (19). To delete the Ikk β^F allele in airway epithelial cells, Ikk $\beta^{F/F}$ mice were crossed with transgenic CC10-Cre^{tg} mice (background strain C57/BL; kindly provided by Jeff Whitsett, University of Cincinnati, Cincinnati) (20, 21), which express two transgenes, one an activator that expresses the reverse tetracycline-responsive transactivator (rtTA) in a Clara cell-specific manner (CC10-rtTA) and the second under control of the tet-operator (tetO), which controls expression of Cre (tetO-Cre; ref. 21). When doxycycline is administered to Ikk $\beta^{F/F}$ /CC10-Cre^{tg} mice, IKK β is deleted in airway epithelium if the progeny expresses both of the transgenes (i.e., CC10-rtTA and tetO-Cre; Fig. 1A and B). Studies using a reporter gene construct have shown that CC10-Cre^{tg}-mediated deletion is relatively rapid, starting within 2 h of administration of tetracycline, and restricted to airway epithelial

cells (21). Clara cells represent $\approx 70\%$ of the adult mouse airway epithelium (22). *In situ* hybridization studies in mouse lung have shown that CC10 is expressed primarily in the airway (bronchi and bronchioles) and not in the alveolar epithelium (23). Pregnant females and litters were maintained on oral doxycycline at all times until they were killed.

Genotyping. To identify CC10-Cre^{tg}/Ikk $\beta^{F/F}$ progeny mice that inherited both CC10-Cre^{tg} transgenes (i.e., CC10-rtTA and tetO-Cre), as well as two Ikk β^F alleles, we used RT-PCR and the primers described in refs. 19–21. To detect the deleted Ikk β^{Δ} allele in airway epithelial cells, an SL μ Cut laser-capture microscope (Molecular Machines and Industries, Knoxville, TN) was used to obtain airway epithelial cells from lung tissue. Lung sections (7.5 μ m) were cut and mounted on microdissection slides, and epithelial cells were captured on thermoplastic caps (Molecular Machines and Industries) by using a 7.5- μ m-diameter laser spot and 50 mW of laser power. We captured $\approx 2,000$ epithelial cells on each cap from an individual mouse lung. The extracted DNA was used for quantitative TaqMan PCR analysis to detect the Ikk β^{Δ} and Ikk β^F alleles by using primers as described in refs. 19 and 24.

Induction of Allergen-Induced Airway Remodeling. The methods that we used to administer OVA allergen to induce airway remodeling in mice were as described in ref. 3. CC10-Cre^{tg}/Ikk $\beta^{\Delta/\Delta}$ mice (i.e., mice in which deletion was induced by doxycycline administration) and littermate IKK β -expressing control mice of the closest genotype (12 mice per group in all described experiments; abbreviated as WT) were used at 8–10 wk of age. Mice were immunized s.c. on days 0, 7, 14, and 21, with 25 μ g of OVA (grade V; Sigma) adsorbed to 1 mg of Alum (Aldrich) in 200 μ l of normal saline. Intranasal OVA challenges (20 ng per 50 μ l in PBS) were conducted on days 27, 29, and 31 under isoflurane (Vedco, St. Joseph, MO) anesthesia and then repeated twice a week for 1 month. A group of age- and sex-matched non-OVA-

challenged *CC10-Cre^{tg}/Ikkβ^{Δ/Δ}* and littermate control mice were sensitized but not challenged with OVA during the 1-month study. Mice were killed at 24 h after the final OVA challenge, and bronchoalveolar lavage (BAL) fluid and lungs were analyzed. All animal experimental protocols were approved by the University of California at San Diego Animal Subjects Committee.

Assessment of Airway Epithelial vs. Nonairway Epithelial Activation of NF-κB. Activation of NF-κB is associated with translocation of dimers, especially those containing RelA, from the cytoplasm to the nucleus. We used immunohistochemistry to determine whether RelA translocated to the nucleus in airway epithelial cells of OVA-challenged mice, using an Ab directed against RelA (Santa Cruz Biotechnology), as well as nuclear counterstaining with SYTOX Green (Molecular Probes) as described in ref. 25. The RelA primary Ab was detected by using a horseradish peroxidase enzyme-labeled secondary Ab (Alexa Fluor 546; red) with tyramide signal amplification (Molecular Probes) according to the manufacturer's instructions. Cells with cytoplasmic RelA had a green nucleus, whereas cells in which RelA was nuclear have a yellow nucleus. Nuclear RelA was determined in both airway epithelial cells as well as in surrounding peribronchial mononuclear cells.

Quantitation of Peribronchial Fibrosis. Lungs were equivalently inflated with an intratracheal injection of a similar volume of 4% paraformaldehyde solution (Sigma) to preserve pulmonary architecture. The area of peribronchial trichrome staining in paraffin embedded lung was outlined and quantified by using a light microscope (DM LS, Leica Microsystems, Buffalo, NY) attached to an image-analysis system (Image-Pro Plus, Media Cybernetics, Bethesda) as described in ref. 3. Results are expressed as the area of trichrome staining per micrometer in length of basement membrane of bronchioles of 150–200 μm in internal diameter. The amount of lung collagen was measured as described in ref. 3 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, Northern Ireland, U.K.). In all experiments, a collagen standard was used for calibration.

Eosinophilic Lung Inflammation. Eosinophil counts were performed on BAL fluid as described in ref. 3. Lung sections were processed for major basic protein (MBP) immunohistochemistry by using an anti-mouse MBP Ab (kindly provided by James Lee, Mayo Clinic, Scottsdale, AZ) as described in ref. 3.

BAL Chemokines. Levels of NF-κB-regulated epithelial-derived chemokines in BAL fluid that mediate eosinophil (e.g., eotaxin-1) or Th2 cell [e.g., thymus- and activation-regulated chemokine (TARC)] recruitment were measured by ELISA (R & D Systems) as described in ref. 3.

Lung TGF-β1 and Peribronchial TGF-β1⁺ Cells. The concentrations of TGF-β1 in lung were assayed by ELISA (R & D Systems) (3). The number of TGF-β1-positive cells per bronchus, as well as the area of epithelium immunostaining positive for TGF-β1 (expressed as micrometers squared of TGF-β1 stained epithelium per micrometer of epithelium) were quantitated as described in ref. 3 in lung sections immunostained with an anti-TGF-β1 Ab.

Quantitation of Airway Mucus Production. Mucus expression in the airway was quantitated by counting the number of periodic acid/Schiff reagent-positive and -negative epithelial cells in individual bronchioles (3).

Airway Smooth Muscle Thickness. The thickness of the airway smooth muscle layer was measured by using an image-analysis system (3, 26).

Statistical Analysis. Results in 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 mean ± SEM. A statistical software package (PRISM, GraphPad, San Diego) was used for the analysis. *P* < 0.05 was considered to be statistically significant.

Results

Genotyping of *CC10-Cre^{tg}/Ikkβ^{Δ/Δ}* Mice and Deletion of *IKKβ*. RT-PCR genotyping showed that crossing *CC10-Cre^{tg}* mice with *Ikkβ^{F/F}* mice produced *CC10-Cre^{tg}/Ikkβ^{Δ/Δ}* progeny that inherited both transgenes (*rtTA*, *tetO*) present in *CC10-Cre^{tg}* mice, as well as both *Ikkβ^F* alleles (Fig. 1C).

CC10-Cre^{tg}/Ikkβ^{F/F} mice, given doxycycline to induce Cre expression and *Ikkβ^F* deletion, were further genotyped to detect deletion of *Ikkβ* exon 3 in airway epithelial cells obtained by laser capture microdissection. TaqMan PCR genotyping studies of airway epithelial cells derived from *CC10-Cre^{tg}/Ikkβ^{Δ/Δ}* mice and littermates showed that *CC10-Cre^{tg}/Ikkβ^{Δ/Δ}* mice, but not the littermates, lost exon-3 sequences in airway epithelial cells as assessed using primers specific for this *Ikkβ* exon (Fig. 1D).

Nuclear Localization of NF-κB After OVA Challenge in Airway Epithelium. Nuclear localization of the RelA NF-κB subunit was assessed in lung sections from *CC10-Cre^{tg}/Ikkβ^{Δ/Δ}* mice and littermate controls. In non-OVA-challenged WT and *CC10-Cre^{tg}/Ikkβ^{Δ/Δ}* mice no nuclear RelA staining could be detected in either lung airway epithelial cells or in peribronchial mononuclear cells (Fig. 1E). By contrast, in WT mice challenged with OVA nuclear staining of RelA was detectable in both airway epithelial cells and peribronchial mononuclear cells. However, in OVA-challenged *CC10-Cre^{tg}/Ikkβ^{Δ/Δ}* mice, RelA was nuclear in peribronchial mononuclear cells but not in most of the airway epithelial cells (Fig. 1E).

Peribronchial Fibrosis in *CC10-Cre^{tg}/Ikkβ^{Δ/Δ}* vs. WT Mice. We used two parameters to quantitate peribronchial fibrosis (namely, the area of peribronchial trichrome staining and total lung collagen). The area of peribronchial trichrome staining in WT mice that were repetitively challenged with OVA was significantly greater than in control non-OVA-challenged WT mice (1.74 ± 0.10 vs. 0.68 ± 0.05 μm²/μm; *P* = 0.001; Figs. 2 and 3A). Baseline levels of peribronchial trichrome staining in non-OVA-challenged mice were similar in *CC10-Cre^{tg}/Ikkβ^{Δ/Δ}* and WT mice. However, *CC10-Cre^{tg}/Ikkβ^{Δ/Δ}* mice that were repetitively challenged with OVA exhibited a significant reduction in levels of peribronchial trichrome staining compared with WT mice (*CC10-Cre^{tg}/Ikkβ^{Δ/Δ}* OVA vs. WT OVA; *P* = 0.001; Figs. 2 and 3A). Levels of lung collagen also were significantly increased in WT mice that were repetitively challenged with OVA ($1,528 \pm 81$ vs. 594 ± 41 μg per lung; WT OVA vs. WT no-OVA; *P* = 0.001). The levels of lung collagen in OVA-challenged *CC10-Cre^{tg}/Ikkβ^{Δ/Δ}* mice were significantly lower relative to OVA-challenged WT mice ($1,027 \pm 75$ vs. $1,528 \pm 81$ μg per lung; *CC10-Cre^{tg}/Ikkβ^{Δ/Δ}* OVA vs. WT OVA; *P* = 0.01).

Mucus Production in *CC10-Cre^{tg}/Ikkβ^{Δ/Δ}* vs. WT Mice. The fraction of the airway epithelium that stained positive with periodic acid/Schiff reagent was increased significantly in WT mice that were repetitively challenged with OVA (40.5 ± 2.1 vs. 1.2 ± 0.1 %; WT OVA vs. WT no OVA; *P* = 0.001; Fig. 3B). There was a

micrometer of epithelium; *CC10-Cre^{tg}/Ikk β ^{$\Delta\Delta$}* OVA vs. WT OVA; no significant difference determined).

Levels of BAL TARC and CD4⁺ Cells. Repetitive OVA challenge in WT mice induced a significant increase in BAL concentrations of TARC ($P = 0.05$ vs. non-OVA WT; Fig. 3G) and the number of peribronchial CD4⁺ cells ($P = 0.05$ vs. non-OVA WT; Fig. 3H). The levels of TARC in BAL fluid were significantly lower in OVA-challenged *CC10-Cre^{tg}/Ikk β ^{$\Delta\Delta$}* mice relative to similarly treated WT mice (*CC10-Cre^{tg}/Ikk β ^{$\Delta\Delta$}* OVA vs. WT OVA; $P = 0.01$; Fig. 3G), and this reduction was associated with a decrease in the number of peribronchial CD4⁺ cells ($P = 0.05$; Fig. 3H).

Peribronchial Smooth Muscle Layer Thickness. The thickness of the peribronchial smooth muscle layer in mice that were repetitively challenged with OVA was similarly increased in WT and *CC10-Cre^{tg}/Ikk β ^{$\Delta\Delta$}* mice (5.1 ± 0.2 vs. 4.9 ± 0.1 μ m; *CC10-Cre^{tg}/Ikk β ^{$\Delta\Delta$}* OVA vs. WT OVA).

Discussion

In this study, we conditionally inactivated the *Ikk β* gene in airway epithelium to examine the role of NF- κ B-regulated genes that are expressed in these cells in allergen-induced airway remodeling including peribronchial fibrosis and mucus expression. Relative to similarly treated WT littermates, *CC10-Cre^{tg}/Ikk β ^{$\Delta\Delta$}* mice, lacking IKK β , only in airway epithelium, exhibited decreased airway epithelial nuclear RelA after repetitive antigen (OVA) challenge and diminished epithelial expression of NF- κ B-regulated chemokines, including eotaxin-1 and TARC, which attract eosinophils and Th2 cells, respectively, into the airway. The number of peribronchial cells expressing TGF- β 1 as well as the levels of BAL TGF- β 1 were also reduced significantly in mice that were deficient in airway epithelial *Ikk β* , suggesting that reduced expression of TGF- β 1 could have contributed to reduced remodeling. We know of no evidence of κ B binding sites in the promoter region of TGF- β 1, suggesting that NF- κ B does not directly regulate expression of this cytokine (27). Also, to precisely identify which NF- κ B-regulated genes in airway epithelium contribute to airway remodeling, studies are needed in which single NF- κ B-regulated genes are reexpressed in airway epithelium of *CC10-Cre^{tg}/Ikk β ^{$\Delta\Delta$}* mice or in which they are specifically inactivated in the epithelium of *Ikk β* expressing mice.

This study provides conclusive evidence that epithelial genes that are directly or indirectly regulated by IKK β and NF- κ B contribute to airway remodeling in a mouse model of asthma. Theoretically, NF- κ B-regulated genes in the airway epithelium could modulate levels of peribronchial fibrosis through epithelial effects on the migration or activation of inflammatory cells, which subsequently influence fibrosis and/or through epithelial effects on collagen deposition by fibroblasts, or collagen removal by other cells. Epithelial cells produce cytokines, such as TGF- β 1 (28), that could act directly on fibroblasts to induce peribronchial fibrosis independent of recruitment of inflammatory cells. As described above, whereas TGF- β 1 is expressed by epithelial cells, its promoter does not contain obvious κ B elements (27), making it unlikely that the reduced levels of lung TGF- β 1 in *CC10-Cre^{tg}/Ikk β ^{$\Delta\Delta$}* mice are due to reduced epithelial TGF- β 1 expression. Our studies show that epithelial levels of TGF- β 1 are not reduced in *CC10-Cre^{tg}/Ikk β ^{$\Delta\Delta$}* mice, whereas there are significant reductions in the numbers of peribronchial TGF- β 1⁺ cells in *CC10-Cre^{tg}/Ikk β ^{$\Delta\Delta$}* mice. The reduced numbers of such cells in *CC10-Cre^{tg}/Ikk β ^{$\Delta\Delta$}* mice reflect the reduced numbers of eosinophils, which express TGF- β 1, that are recruited to the airway. We have recently shown that eosinophils expressing

TGF- β 1 are important contributors to allergen-induced peribronchial fibrosis in studies using IL-5-deficient mice (3). The >90% decrease in eosinophil numbers in such mice was associated with a significant reduction in levels of lung TGF- β 1 and peribronchial fibrosis suggesting that depletion of eosinophils that express TGF- β 1 contributed significantly to the development of peribronchial fibrosis (3). Similar observations were made in human subjects with asthma in whom depletion of eosinophils with anti-IL-5 therapy significantly reduced subepithelial deposition of extracellular matrix proteins, the number of BAL eosinophils expressing TGF- β 1, and total BAL TGF- β 1 (5). The importance of TGF- β to peribronchial fibrosis is suggested also by recent studies showing that administration of anti-TGF- β Ab to mice subjected to repetitive allergen challenge inhibits allergen-induced peribronchial fibrosis (29).

The reduction in levels of eosinophils in the airway of *CC10-Cre^{tg}/Ikk β ^{$\Delta\Delta$}* mice is likely to be due to reductions in levels of chemokines such as eotaxin-1, which are expressed by airway epithelial cells. There is considerable evidence that NF- κ B may be involved in regulation of eotaxin-1 expression. The eotaxin-1 promoter contains κ B sites in both mice (30) and humans (31). *In vitro* studies using epithelial cells have shown that a κ B site in the eotaxin promoter is critical for its induction by TNF (32), whereas mice that are deficient in the NF- κ B p50 subunit do not express eotaxin after acute OVA challenge (13). Also, in these mice the absence of eosinophilic inflammation after acute OVA challenge was correlated with diminished IL-5 and eotaxin gene expression (13). Although in our study selective inhibition of NF- κ B activation in airway epithelium significantly inhibited eotaxin-1 expression, it had a marginal effect on IL-5 expression, showing that the airway epithelium is a major source for eotaxin-1, but not IL-5, and that IKK β -dependent NF- κ B transcription factors are required for eotaxin-1 expression. Thus, our detection of significantly reduced levels of eotaxin-1 and TARC in BAL fluid of *CC10-Cre^{tg}/Ikk β ^{$\Delta\Delta$}* mice suggests that IKK β regulates epithelial expression of chemoattractants for eosinophils and CD4⁺ cells, presumably through NF- κ B in the remodeled airway. The mediator induced by repetitive allergen challenge that activates NF- κ B in airway epithelial cells *in vivo* is unknown, but possibilities include TNF (12) or other inflammatory cytokines (12), which are expressed after allergen challenge and are able to activate the IKK complex (10).

In this study, we found evidence supporting the contribution of airway epithelial genes regulated by NF- κ B to specific structural aspects of allergen-induced airway remodeling. In particular, we have shown that a subset of airway epithelial genes, whose elevated expression during chronic airway inflammation requires activation of transcription factor NF- κ B through IKK β , have a key role in mediating allergen-induced peribronchial fibrosis and mucus expression. Although these NF- κ B regulated epithelial genes play an important role in mediating allergen-induced peribronchial fibrosis and mucus expression, they do not participate in other aspects of airway remodeling, such as increased smooth muscle thickness. The finding that NF- κ B regulated genes in airway epithelium contribute to allergen-induced peribronchial fibrosis and mucus expression raises the possibility that an inhaled NF- κ B antagonist, or an IKK β inhibitor, may represent an effective therapeutic strategy for prevention of fibrosis and elevated mucus production in chronic asthma or other lung diseases such as chronic obstructive pulmonary disease while at the same time limiting systemic NF- κ B inhibitory effects, which could contribute to toxicity or cause an immunodeficiency.

This work was supported by National Institutes of Health Grant AI38425 (to D.H.B.) and a Sandler grant (to M.K.).

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