

Role of Interleukin-5 and Eosinophils in Allergen-Induced Airway Remodeling in Mice

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Asthma is a chronic inflammatory disease characterized by variable bronchial obstruction, hyperresponsiveness, and by tissue damage known as airway remodeling. In the present study we demonstrate that interleukin (IL)-5 plays an obligatory role in the airway remodeling observed in experimental asthma. BALB/c mice sensitized by intraperitoneal injections of ovalbumin and exposed daily to aerosol of ovalbumin for up to 3 wk, develop eosinophilic infiltration of the bronchi and subepithelial and peribronchial fibrosis. The lesions are associated with increased amounts of hydroxyproline in the lungs and elevated levels of eosinophils and transforming growth factor (TGF)- β 1 in the bronchoalveolar lavage fluid. After 1 wk of allergen challenge, TGF- β is mainly produced by eosinophils accumulated in the peribronchial and perivascular lesions. At a later stage of the disease, the main source of TGF- β is myofibroblasts, identified by α -smooth muscle actin mAb. We show that all these lesions, including fibrosis, are abolished in sensitized and allergen-exposed IL-5 receptor-null mice, whereas they are markedly accentuated in IL-5 transgenic animals. More importantly, treatment of wild-type mice with neutralizing anti-IL-5 antibody, administered before each allergen challenge, almost completely prevented subepithelial and peribronchial fibrosis. These findings demonstrated that eosinophils are involved in allergen-induced subepithelial and peribronchial fibrosis probably by producing a fibrogenic factor, TGF- β 1.

Bronchial asthma is a chronic inflammatory disorder, characterized by variable and reversible bronchial obstruction, airway eosinophilic inflammation, and bronchial hyperresponsiveness (1). However, patients with chronic asthma develop irreversible alterations of pulmonary function despite appropriate and aggressive anti-inflammatory therapy (2, 3). These alterations result from the structural changes of the airways, known as airway remodeling, characterized by goblet cell hyperplasia, subepithelial fibrosis, and smooth muscle hypertrophy (4). The precise mechanisms leading to airway remodeling are still unknown, but it is thought to result from an injury-repair response driven by several mediators derived from the inflammatory cells.

Eosinophils are thought to be principal inflammatory cells in the pathophysiology of the disease, through the release of lipid mediators, cytokines, and cytotoxic proteins (5). Eosinophils also produce fibrogenic factors, such as transforming growth factor- β 1 (TGF- β 1) (6) and platelet-derived growth factor (7). Eosinophils induce fibroblast proliferation (8) and accumulate

in the lesional sites of various fibrotic disorders (9). These observations suggest that eosinophils may also play an important role in the airway remodeling of patients with asthma; however, this hypothesis has not been fully investigated *in vivo* and the results are still controversial (10–12). Moreover, role of eosinophil-derived TGF- β 1 in the development of airway remodeling caused by allergen challenge has not been fully examined.

Recently, we have established a mouse model of allergic asthma in which sensitized animals are exposed daily to allergen aerosol for three consecutive weeks (13–15). As a result, mice develop a typical Th2 response leading to bronchial hyperresponsiveness to cholinergic stimuli, eosinophilic inflammation, goblet cell hyperplasia, and subepithelial fibrosis (13–15). Allergen-induced airway remodeling revealed to be Th2-dependent and closely associated with the intensity of airway eosinophil infiltration (14, 15). Moreover, there was a clear correlation between subepithelial fibrosis and both the levels of TGF- β 1 and the numbers of eosinophils in bronchoalveolar lavage fluid (BALF) (13).

In the present study, we analyzed the role of interleukin (IL)-5 and eosinophils in the development of airway remodeling, by using genetically manipulated mice lacking IL-5 receptor α chain (IL-5R α KO), or mice transgenic for IL-5 (IL-5Tg). In addition, we have examined the role of IL-5 in airway remodeling by treating allergic wild-type animals with a neutralizing Ab to IL-5.

Materials and Methods

Animals

Seven-week-old female BALB/c mice (Japan SLC, Shizuoka, Japan), IL-5 transgenic mice (IL-5Tg; BALB/c background; 16), IL-5 receptor α chain gene KO mice (IL-5R α KO; 129 Ola $\times$ BALB/c background; backcross to BALB/c five times [N5]; 17) and age-matched wild-type animals were used. Experiments were undertaken following the guidelines for the care and use of experimental animals of the Japanese Association for Laboratory Animals Science in 1987.

Agents

The following drugs and chemicals were purchased commercially and used: ovalbumin (OVA; Seikagaku Kogyo, Tokyo, Japan), bovine serum albumin (Seikagaku Kogyo), Türk solution (Wako Pure Chemical Industries, Ltd., Osaka, Japan), sodium pentobarbitone (Abbott Lab., Chicago, IL), EDTA-2Na (Nacalai Tesque, Kyoto, Japan), Diff-Quick solution (International Reagent Corp., Ltd., Kobe, Japan), an mAb against human IL-5 (5A5, mouse IgG1), and hydroxy-L-proline (Nacalai Tesque).

Sensitization and Antigen Challenge

Experiments were performed as reported previously (14, 15). Mice were actively sensitized by intraperitoneal injections of 50 μ g OVA with 1 mg alum on Days 0 and 12. Starting on Day 22, they were exposed to OVA (1% wt/vol diluted in sterile physiological saline) for 30 min every day for 3 consecutive wks. As a negative control, animals were

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Abbreviations: bronchoalveolar lavage, BAL; BAL fluid, BALF; hydroxyproline, HP; interleukin, IL; ovalbumin, OVA; phosphate-buffered saline, PBS; receptor α chain, R α ; transgenic, Tg; transforming growth factor, TGF.

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injected with saline or OVA plus alum and exposed to saline in a similar manner. BAL, as well as biochemical and histologic examination, were performed 24 h after the final antigen challenge.

Treatment of Anti-IL-5 mAb

The mAb against human IL-5 (5A5, mouse IgG1) was purified from ascites by sequential (NH₄)₂SO₄ precipitation and protein A affinity chromatography, dialyzed against phosphate-buffered saline (PBS), and kept at -80°C until use. The mAb (0.5 or 1 mg/animal) was treated by intraperitoneal injection 1 h before every antigen inhalation. Control Ab (mouse IgG1) was treated in a similar manner. In a preliminary experiment, we confirmed the efficacy of the mAb to neutralize mouse IL-5 using Th2 cells (D10. G4. 1: mouse Th2 cells clone)-mediated peritoneal eosinophilia.

BAL

To evaluate airway inflammation, we examined the accumulation of inflammatory cells in BALF. Experiments were performed according to previously described methods (14, 15). Animals were killed with an intraperitoneal injection of sodium pentobarbitone (100 mg/kg). The trachea was cannulated and the left bronchi were tied for histologic examination. Then, the right air lumen was washed 4 times with 0.5-ml calcium- and magnesium-free PBS containing 0.1% bovine serum albumin and 0.05 mM EDTA-2Na. This procedure was repeated three times (total volume; 1.3 ml, recovery > 85%). BALF from each animal was pooled in a plastic tube, cooled on ice and centrifuged ($150 \times g$) at 4°C for 10 min. Cell pellets were resuspended in the same buffer (0.5 ml). BALF was stained with Türk solution, and the number of nucleated cells was counted in a Burker chamber. A differential count was made on a smear prepared with a cytocentrifuge (Cytospin II; Shandon, Cheshire, UK) and stained with Diff-Quick solution (based on standard morphologic criteria) of at least 300 cells (magnification $\times 400$). The supernatant of BALF was stored at -30°C for determination of cytokine production.

Cytokine Levels in BALF

The TGF- β 1 content in BALF was also measured using ELISA (Genzyme Tecne, Minneapolis, MN), which can detect mouse TGF- β 1 protein, because of the high homology of TGF- β 1 across species. The assay detects only the active form of TGF- β 1. Each sample was directly measured for the detection of the active form or was activated before measuring according to the manufacturer's recommendations, for the detection of total amount of TGF- β 1. The detection limit was 7 pg/ml.

Measurement of Hydroxyproline Content in the Right Lungs

Whole collagen content of the right lung was evaluated by determining hydroxyproline (HP) content as described previously (14, 15). Briefly, after recovery of BALF, the right lung lobes were removed and cut into sections (1 mm thick). The chopped lungs were dried with acetone. Then, the dried lung samples were hydrolyzed with 2 ml of 6N HCl at 120°C for 24 h in sealed glass tubes. The amount of HP in the hydrolysate was measured according to Kivirikko and coworkers (18). Authentic HP (hydroxy-L-proline) was used to establish a standard curve.

Histopathologic Study

The left lungs were distended with 10% buffered formalin via the trachea (10 cm H₂O) for 30 min, and then excised and immersed in the fresh fixative for 24 h. Tissues were sliced and embedded in paraffin, and 6- μm sections were stained with hematoxylin and eosin and Masson-Trichrome for light microscopic examination. Section analyses, described below in detail, were performed in a blind fashion, and slides were presented in random order for each examination.

Masson-trichrome stained sections were used for assessment of sub-epithelial fibrosis using a Leica image analysis system (Leica, Cambridge, UK) as described previously (15). Briefly, two to four specimens of the Masson-trichrome-stained histologic preparations of the left lobe, in which the total length of the epithelial basement membrane of the bronchioles was 1.0–2.5 mm, were selected and the fibrotic area (stained in blue) beneath the basement membrane at 20 μm depth was measured. The

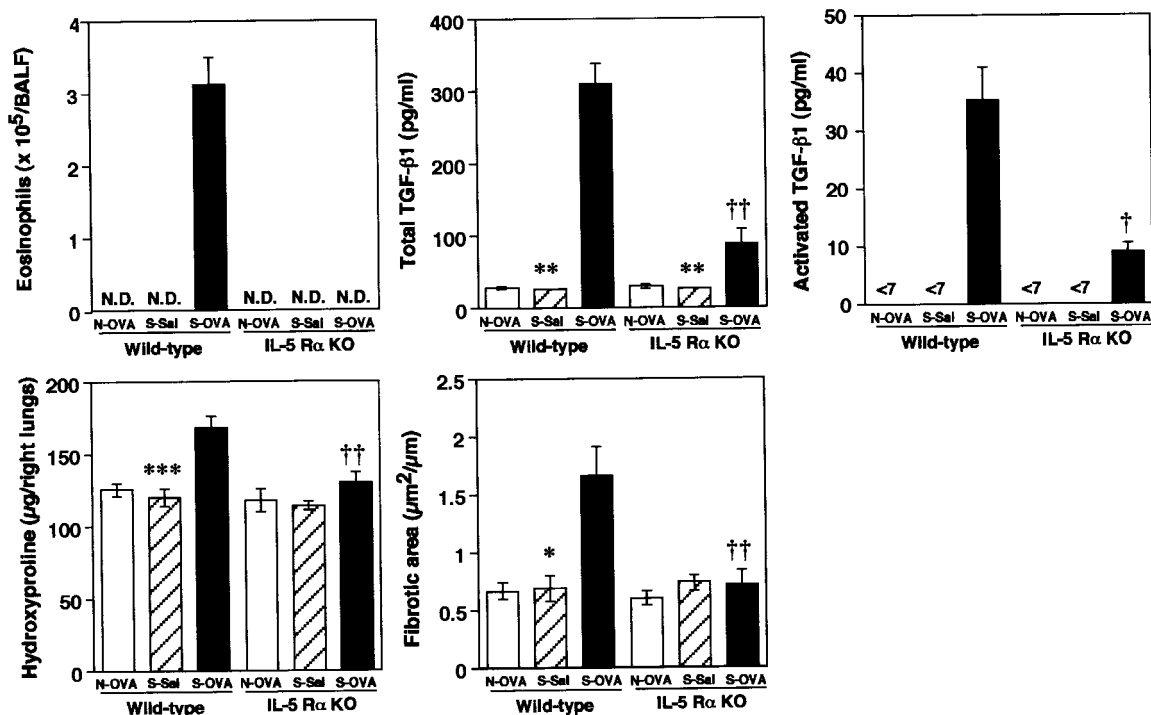


Figure 1. Effect of IL-5 α gene deficiency on allergen-induced increases in the numbers of eosinophils in BALF, TGF- β 1 production in BALF, and the amount of HP in the right lungs in sensitized BALB/c background mice. Repeated allergen challenge induced increases in the numbers of eosinophils and the level of TGF- β 1 in BALF and the amount of HP in the right lung in sensitized wild-type animals. In contrast, the deficiency of IL-5 signaling through IL-5 α clearly inhibited these parameters. Values represent the mean $\pm$ SEM of 5–8 mice in each group. N.D., not detected; N, nonsensitized; OVA, ovalbumin-exposed; S, sensitized; Sal, saline-exposed. $^{**}P < 0.01$, $^{***}P < 0.001$ (versus S-OVA group); $^{\dagger}P < 0.01$ (versus wild-type).

mean scores of the fibrotic area divided by basement membrane length in 2–4 preparations of one mouse were calculated, then the mean scores of subepithelial fibrosis were calculated in each group.

Immunohistochemistry

To identify the cellular source of TGF- β 1 within the airway wall, immunostaining was performed using avidin–biotin peroxidase complex method. The left lungs were distended with 10% buffered formalin via the trachea (10 cm H₂O) for 30 min, and then excised and immersed in the fresh fixative for 24 h. Tissues were sliced and embedded in paraffin, and 4- μ m sections were treated with 0.3% hydrogen peroxide-methanol and normal goat serum for blocking nonspecific binding and endogenous peroxidase activity. Sections were washed with PBS and stained with a polyclonal Ab against TGF- β (SC-146, rabbit IgG; Santa Cruz, Santa Cruz, CA), which recognize a peptide mapping at the carboxy terminus of the precursor form of TGF- β 1 of human origin (identical to corresponding mouse sequences). Slides were then washed and incubated with biotinylated goat polyclonal anti-rabbit immunoglobulin (DakoCytomation Co. Ltd., Kyoto, Japan). Color development was conducted using streptavidin-labeled peroxidase (Nichirei Corporation, Tokyo, Japan) and 3,3'-diaminobenzidine tetrahydrochloride (Histofine; Nichirei) as a chromogen. As a control, rabbit IgG was used.

To identify the cells positive for α -smooth muscle actin, the sections were treated with 0.3% hydrogen peroxide-methanol. After washing with PBS, sections were stained with peroxidase-labeled anti- α -smooth muscle actin Ab (1A4; DakoCytomation Co. Ltd.). Color development was conducted using 3,3'-diaminobenzidine tetrahydrochloride (Histofine; Nichirei) as a chromogen.

Statistical Analysis

Values are presented as the mean $\pm$ SEM. Statistical significance between two groups was estimated using the two-tailed Student's *t* test or the Mann-Whitney *U* test after the variances of the data were evaluated with *F*-test. To define statistically significant differences among control animals and mAb-treated animals, the data were subjected to Bartlett's analysis followed by a parametric or a non-parametric

Dunnnett's multiple range test. *P* values < 0.05 were considered to be significant.

Results

Effect of the Deficiency of IL-5R α

To clarify whether the deficiency of IL-5 signaling influenced the development of allergen-induced airway remodeling, we examined the accumulation of eosinophils in BALF, the production of a fibrogenic factor, TGF- β 1, in BALF, the amount of HP in the right lung tissues, and the fibrotic area around the airways in IL-5R α KO mice compared with those of wild-type mice. As shown in Figure 1, repeated allergen inhalation induced the significant increases in the numbers of eosinophils and total and activated TGF- β 1 production in BALF, the amount of HP in the lungs, and the fibrotic area around the airways in sensitized wild-type mice. In contrast, the deficiency of IL-5 signaling through IL-5R α clearly abolished allergen-induced airway eosinophilia and significantly attenuated the increased levels of both total and activated TGF- β 1 and the fibrotic changes around the airways (*P* < 0.01).

Effect of Systemic Overexpression of IL-5

We next investigated the effect of systemic overexpression of IL-5 on subepithelial and peribronchial fibrosis caused by chronic allergen challenge using IL-5Tg mice compared with BALB/c mice. As expected, allergen-induced increases in the numbers of eosinophils in BALF was significantly potentiated in IL-5Tg mice compared with BALB/c mice (*P* < 0.05) (Figure 2). In addition, the level of total but not activated TGF- β 1 in the airways (*P* < 0.05), the HP levels, and the fibrotic lesions (*P* < 0.01) were significantly augmented in IL-5Tg mice.

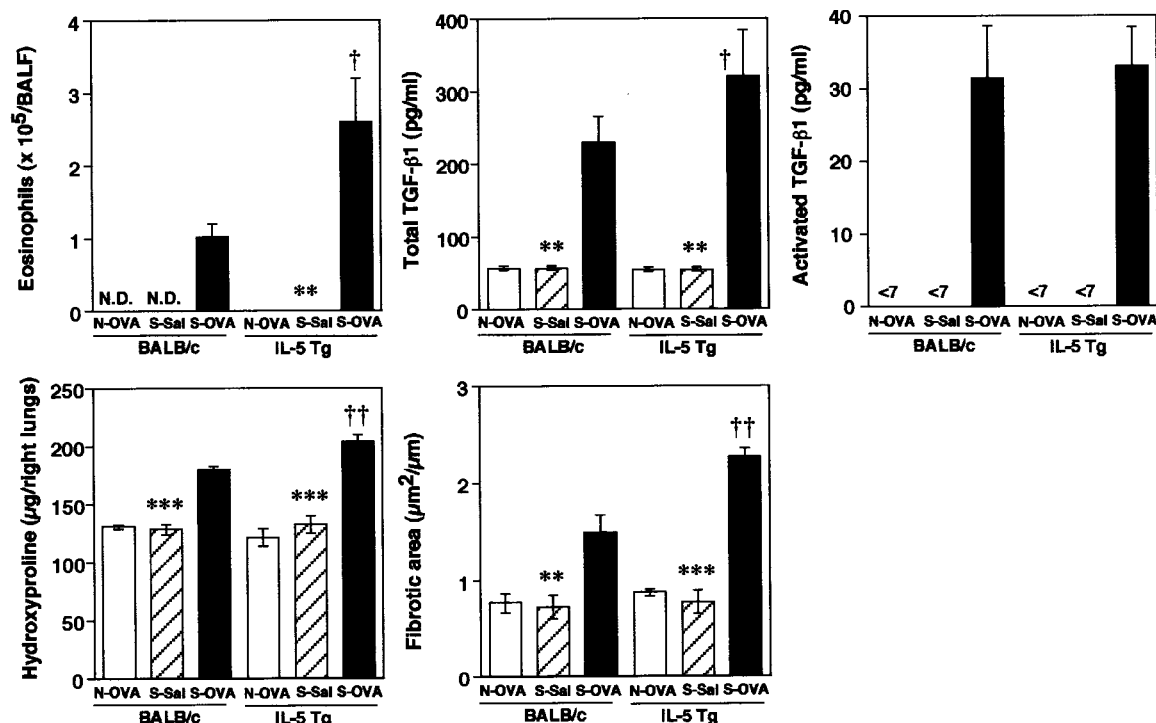


Figure 2. Effect of systemic overexpression of IL-5 on allergen-induced increases in the numbers of eosinophils in BALF, TGF- β 1 production in BALF, and the amount of HP in the right lungs in sensitized BALB/c mice. The overproduction of IL-5 significantly augmented airway eosinophilia, TGF- β 1 production, and the amount of HP. Values represent the mean $\pm$ SEM of 5–8 mice in each group. N.D., not detected; N, nonsensitized; OVA, ovalbumin-exposed; S, sensitized; Sal, saline-exposed. ***P* < 0.01, ****P* < 0.001 (versus S-OVA group); †*P* < 0.05, ††*P* < 0.01 (versus BALB/c).

Effect of a Neutralizing mAb against IL-5 during Allergen Challenge

The implication of IL-5 in allergic airway remodeling was confirmed by the treatment of wild-type animals with neutralizing anti-IL-5 mAb before each allergen challenge. As seen in Figure 3, this treatment significantly inhibited, in a dose-dependent manner, allergen-induced airway eosinophilia, the increases in total but not activated TGF- β 1 production in BALF and the subepithelial and peribronchial fibrosis, and normalized the HP level ($P < 0.01$).

Histopathologic Examination

Figure 4 shows the representative sections of each group stained with Masson-Trichrome for detection of connective tissue. The quantitative findings of the subepithelial and peribronchial fibrosis are shown in Figures 1–3 as described above.

As shown in Figure 4B, OVA-exposed sensitized BALB/c mice displayed increased in fibrotic area beneath the basement membrane of the bronchi compared with saline-exposed animals (Figure 4A). In contrast, the deposition of connective tissue was clearly abrogated in IL-5R α KO animals (Figure 4C), and markedly increased in IL-5Tg mice (Figure 4D). Moreover, the fibrotic changes were significantly attenuated by treatment of wild-type animals with neutralizing anti-IL-5 mAb (Figure 4F), whereas the administration of mouse IgG1 did not affect these structural changes (Figure 4E).

Immunohistochemical Analysis

To determine the cellular source of TGF- β , lung sections were stained with anti-TGF- β mAb at Day 28 (after 1 wk of allergen challenge, Figure 5B), Day 35 (after 2 wk of allergen challenge, Figure 5D) and Day 42 (after 3 wk of allergen challenge, Figure 5F). Saline-exposed mice displayed few TGF- β -producing cells during the period of exposure (Figures 5A, 5C, and 5E). After

1 wk, lung sections of OVA-exposed mice showed significant inflammatory infiltrates around the bronchi and blood vessels. Inflammatory cells, including mononuclear cells, alveolar macrophages, and especially eosinophils, produced TGF- β (Figures 5B, 6B, and 6D). In contrast, mesenchymal cells around the blood vessels and the airways as well as alveolar epithelial cells became positive for TGF- β on Days 35 and 42, and the intensity of the staining was related to the duration of allergen challenge (Figures 5D and 5F). Moreover, the cells around the airways and the vessels were myofibroblasts, as identified by staining with an Ab specific to α -smooth muscle actin (Figures 6F and 6G).

Discussion

The present study demonstrates the obligatory role of IL-5 in the airway remodeling observed in an experimental model of chronic asthma. The development of allergen-induced subepithelial and peribronchial fibrosis was abolished in IL-5R α KO mice, markedly attenuated in wild-type animals treated with a neutralizing mAb to IL-5, and strongly increased in IL-5Tg mice. Subepithelial fibrosis, a typical feature of airway remodeling in patients with asthma, results from the deposition of collagen type III, I, V, and fibronectin in the reticular layer underneath the basement membrane (19). In keeping with recent findings, our observations suggest that the collagen deposition was related to the airway eosinophilic inflammation, probably through TGF- β 1 production. Within the first week of allergen challenge, TGF- β 1 was mainly produced by eosinophils, whereas after 2–3 wk, myofibroblasts, identified as α -smooth muscle actin-positive cells, were the major source of this factor.

TGF- β 1 was reported to be an important factor in the pathogenesis of fibrosis, because of its ability to stimulate the production

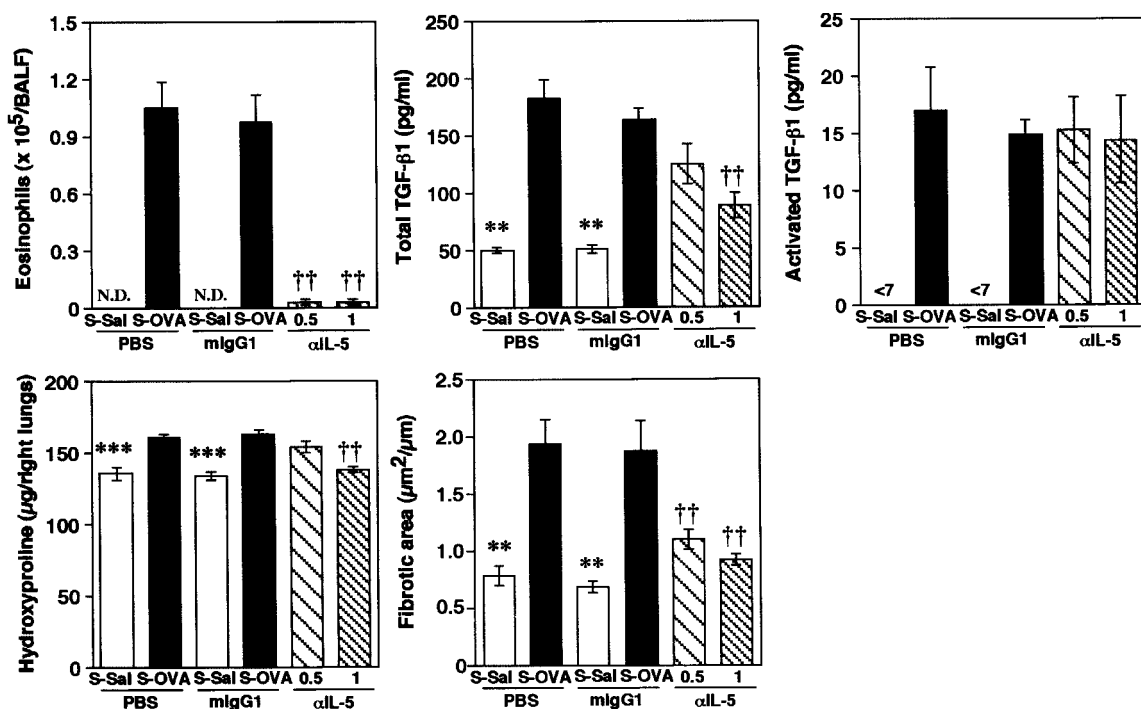


Figure 3. Effect of a neutralizing mAb against IL-5 (α IL-5; 0.5 or 1 mg/mouse) on allergen-induced increases in the numbers of eosinophils in BALF, TGF- β 1 production in BALF, and the amount of HP in the right lungs in sensitized BALB/c mice. The neutralization of IL-5 function with the mAb during allergen challenge clearly inhibited these parameters in a dose-dependent manner. Values represent the mean $\pm$ SEM of 6–7 mice in each group. mIgG1, mouse IgG1; N.D., not detected; OVA, ovalbumin-exposed; S, sensitized; Sal, saline-exposed. ** $P < 0.01$, *** $P < 0.001$ (versus S-OVA group); †† $P < 0.01$ (versus mIgG1-S-OVA).

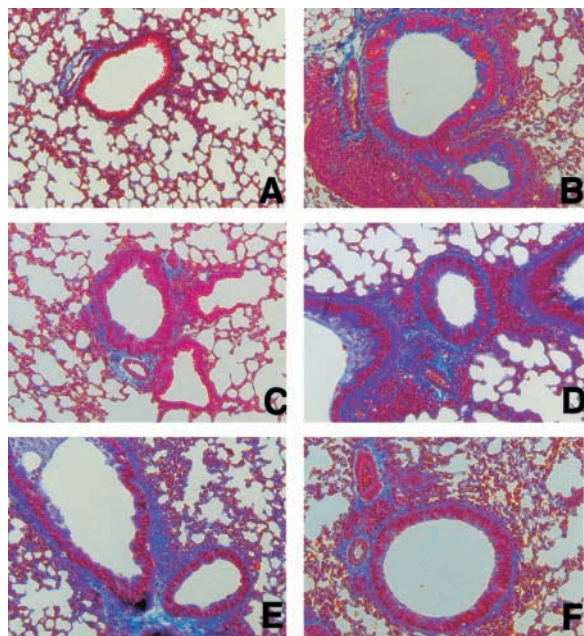


Figure 4. Subepithelial and peribronchial fibrosis (blue) after 3 wk of allergen challenge. Histologic analysis of lung sections stained with Masson-trichrome ($\times 100$) 24 h after the final antigen challenge of sensitized mice. Allergen-induced subepithelial and peribronchial fibrosis (**B** and **E**) was clearly attenuated in IL-5R α KO mice (**C**) and in wild-type mice treated with neutralizing mAb against IL-5 during allergen challenge (**F**). By contrast, massive fibrotic changes around the bronchi and the blood vessels were observed in IL-5Tg animals (**D**). (**A**) Sensitized and saline-challenged BALB/c mice; (**B**) sensitized and OVA-challenged (S-OA) BALB/c mice; (**C**) S-OVA IL-5R α KO mice; (**D**) S-OVA IL-5Tg mice; (**E**) S-OVA wild-type mice treated with murine IgG1; (**F**) S-OVA wild-type treated with neutralizing mAb against IL-5 (1 mg/mouse).

of extracellular matrix proteins and to inhibit the formation of extracellular proteases (20). In fact, a large amount of TGF- β 1 is found in clinical specimens obtained from fibrotic diseases, such as early keloid skin lesions, scleroderma, and pulmonary fibrosis (20). Furthermore, inhibition of TGF- β 1 prevents lung and liver fibrosis in animal models (20). The expression of TGF- β 1 is increased in BALF and biopsy specimens of patients with asthma, and its expression levels correlate with the severity of the disease and the degree of subepithelial fibrosis (21). In addition, Kobayashi and coworkers clearly demonstrated that mouse eosinophils from IL-5 Tg mice can produce TGF- β 1 spontaneously, and that the production was enhanced in the presence of IL-5 or IL-3 (22). Thus, IL-5 produced in the airways after repeated allergen challenge may promote eosinophils to produce TGF- β 1. Taken together, the present findings demonstrated that IL-5 signaling recruits eosinophils that induce subepithelial and peribronchial fibrosis associated with the production of TGF- β at the early stage of airway inflammation, and indirectly, probably accompanied by the transformation of fibroblasts into myofibroblasts producing TGF- β 1 at a later stage of the experimental disease.

TGF- β 1 is produced in an inactive form that requires activation before it can exert a biological effect. In the present study, total amount of TGF- β 1 in BALF was associated with the numbers of eosinophils in BALF. In contrast, the amount of the active form was $\sim 10\%$ of total TGF- β 1 in OVA-inhaled mice, and it was not associated with fibrotic area or eosinophil counts in the airways. These findings suggested that total TGF- β 1 pro-

duction is clearly dependent on airway eosinophilia and that eosinophils by themselves are not involved in its activation in our model, although the dissociation between the amount of active form TGF- β 1 and the degree of structural changes could not be explained, therefore, further experiments will be needed.

Myofibroblasts have been identified as a key component of active fibrotic lesions and are thought to be responsible for collagen production (23). Myofibroblast hyperplasia is a typical feature of asthma and its intensity is correlated with the amount of subepithelial collagen deposition (24). Here, we found myofibroblasts hyperplasia after repeated allergen challenges for 3 wk. At this late stage of the experimental disease, myofibroblasts were the main cellular source of TGF- β 1, instead of eosinophils that were less abundant, possibly because of apoptotic cell death induced by TGF- β 1 (25). *In vivo* and *in vitro* observations have strongly suggested that TGF- β 1 itself is responsible for the differentiation of perivascular adventitial fibroblasts into myofibroblasts (26). Moreover, Phipps and colleagues clearly demonstrated that skin fibroblasts differentiate into myofibroblasts upon coculture with eosinophils, through the production of TGF- β 1 from eosinophils (27). Morishima and coworkers also reported that myofibroblasts induced by TGF- β 1 are functionally more active in producing collagen than resting fibroblasts *in vitro* (28). These findings indicate that accumulated and/or differentiated myofibroblasts around the airways after repeated allergen challenge could be responsible in subepithelial and peribronchial fibrosis in our model. In contrast, a more recent study has demonstrated that fibrocytes, a population of circulating cells, are precursors of myofibroblasts and are involved in the genesis of subepithelial fibrosis in individuals with allergic asthma (29). Thus, it is also possible that circulating fibrocytes can migrate into the injured tissue and differentiate into myofibroblasts in this model.

TGF- β 1 is also reported to be produced by airway epithelial cells (30, 31). Pelton and colleagues demonstrated that both mRNA and protein expression for all three isoforms of TGF- β (1–3) are localized in the murine epithelium (30). More importantly, Morishima and associates (28) and Zhang colleagues (31) clearly demonstrated that TGF- β 1 secreted by airway epithelial cells is involved in myofibroblasts induction and proliferation *in vitro*. In the present study, we detected little TGF- β 1 signals in bronchial and peribronchial epithelial cells after repeated allergen challenge. Furthermore, we could not detect both latent and active form of TGF- β 2 in BALF at any time point during allergen challenge (data not shown). Therefore, TGF- β 1 seems to be the major isoform produced in the airways in our model.

In addition to its fibrogenic properties described above, TGF- β 1 is also a multifunctional cytokine with potent anti-inflammatory activities (20). In fact, this cytokine was recently shown to be a potent negative regulator of bronchial hyperresponsiveness and airway inflammation in experimental asthma (32). For example, the transfer of Ag-specific Th cells engineered *in vitro* to express TGF- β 1 abolished bronchial hyperresponsiveness and airway inflammation induced by Ag-specific Th2 cells (33). Conversely, allergen-induced airway eosinophilic inflammation was markedly enhanced in Tg mice expressing a dominant-negative TGF- β receptor on their T cells (34). Therefore, therapeutic inhibition of TGF- β 1 could not attenuate airway remodeling, because it would augment the airway inflammation generating the fibrotic healing response.

Regarding as the role of IL-5 in the development of allergen-induced airway remodeling, Blyth and coworkers (10) and Trifileff and colleagues (12) have reported that IL-5 is a critical factor in allergen-induced subepithelial fibrosis using a neutralizing antibody or IL-5 gene-deficient mice, respectively. Our present findings are supported by their data, although they did not provide the relation between the numbers of eosinophils and the

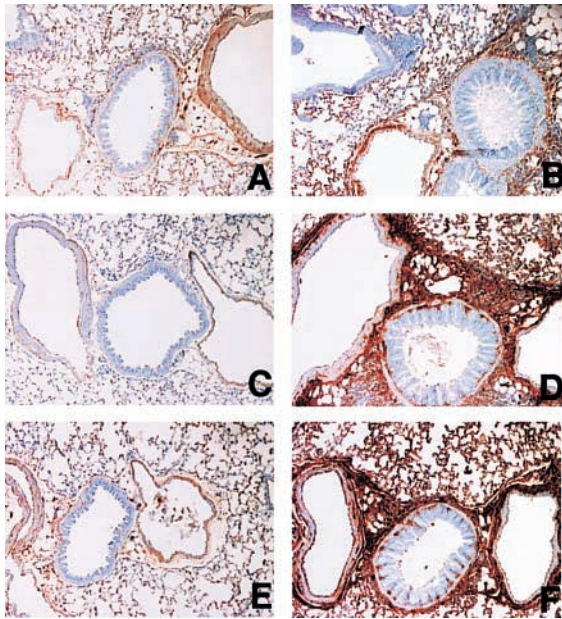


Figure 5. Immunohistochemical staining of lung sections with anti-TGF- β mAb after aerosol challenge for 1 wk (Day 28, *B*), 2 wk (Day 35, *D*), and 3 wk (Day 42, *F*) compared with saline-exposed animals (*A*, *C*, and *E*, respectively) ($\times 100$). Saline-exposed animals appreciated few cells produced TGF- β during the period of exposure (*A*, *C*, and *E*). After 1 wk, inflammatory cells around the airways and the blood vessels produced TGF- β in the lung section of OVA-inhaled animals (*B*). In contrast, mesenchymal cells around the airways and the blood vessels as well as alveolar epithelial cells became positive for TGF- β at the late stage, i.e., on Days 35 (*D*) and 42 (*F*), and the intensity was dependent on the duration of allergen challenge.

production of TGF- β 1 in the airways. In contrast, Foster and associates clearly demonstrated that epithelial and fibrotic changes after chronic allergen inhalation were not dependent on IL-5 in sensitized BALB/c mice. The discrepancy between their data and our present findings may be due to the differences in the experimental protocol. In their model, mice were immunized with OVA with alum, and then exposed OVA (2.5% wt/vol) 3 d/wk for 6 wk, whereas sensitized mice were exposed OVA (1% wt/vol) every day for three consecutive weeks in the present study. Especially, the frequency and concentration of allergen challenge may influence the mechanisms which cells and/or functional molecules are involved in the development of asthma-like responses as reported (35), although similar data were observed with the treatment of anti-CD4 mAb in both laboratories (14, 36).

We recognize some limitations in the present study. First, the mechanisms by which myofibroblasts accumulate in the site of inflammation are not clear. Second, it is also unknown how IL-5/eosinophils participate in myofibroblast hyperplasia around the airways. As described above, there is a possibility that the origin of myofibroblasts is extrapulmonary in origin and is especially derived from bone marrow (29). Therefore, further experiments will be needed to clarify first the origin of these cells in our model using the transgenic mice expressing green fluorescent protein.

Recently, clinical trials of a humanized antibody to IL-5 in mild or severe asthma were performed (37, 38). The antibody is shown to have a potential to inhibit the increases in the numbers of eosinophils in sputum, but asthmatic symptoms in patients were not affected at all. Now, the interpretations of the

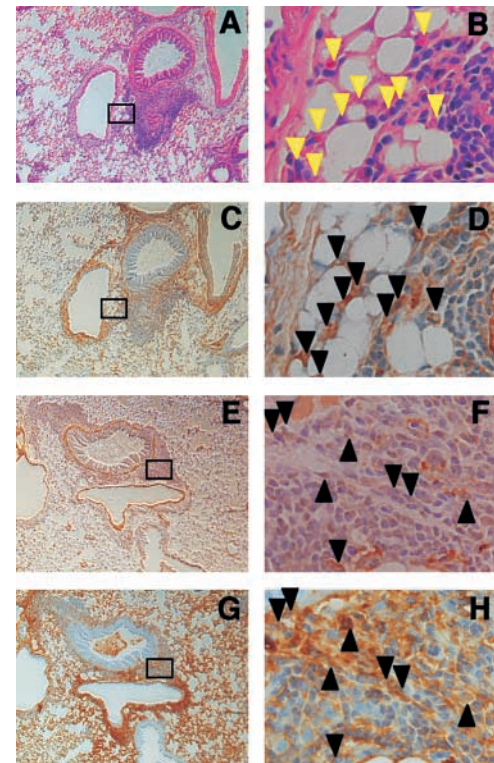


Figure 6. Immunohistochemical staining of lung section with hematoxylin and eosin (*A* and *B*) and anti-TGF- β Ab (*C* and *D*), at Day 28 (after allergen challenge for 1 wk). Arrowheads are eosinophils, which produced TGF- β around the airways and the blood vessels (*B* and *D*). Panels *E*–*H* correspond to tissue sections obtained after 3 wk of allergen challenge (Day 42) and stained with anti- α -smooth muscle actin Ab (*E* and *F*) or anti-TGF- β Ab (*G* and *H*). Arrowheads are myofibroblasts, located around the airways and the blood vessels, producing TGF- β and stained with anti- α -smooth muscle actin Ab. Original magnification: $\times 100$ for *A*, *C*, *E*, and *G*, $\times 1,000$ for *B*, *D*, *F*, and *H*, respectively.

disappointing results of their studies (37, 38) are still discussed (39, 40). Although there has been no information available on the role of eosinophils and IL-5 in airway remodeling of the disease and the present model did not demonstrate the complete features of airway remodeling in asthma, the present findings raise the possibility that the inhibition of the function of IL-5 and/or eosinophils may have a therapeutic approach to prevent the airway remodeling, especially subepithelial fibrosis, in allergic asthma.

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