Manipulation of Allergen-Induced Airway Remodeling by Treatment with Anti-TGF-β Antibody: Effect on the Smad Signaling Pathway

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Airway inflammation and remodeling are important pathophysiologic features of chronic asthma. Previously, we have developed a mouse model of prolonged allergen challenge which exhibits many characteristics of chronic asthma such as goblet cell hyperplasia and subepithelial collagen deposition, in association with an increase in lung expression of the profibrotic mediator, TGF-β. The aim of this study was to determine the effects of blockade of TGF-β on the development of airway inflammation and remodeling using our murine model of prolonged allergen challenge. Importantly anti-TGF-β Ab was administered therapeutically, with dosing starting after the onset of established eosinophilic airway inflammation. Therapeutic treatment of mice with anti-TGF-β Ab significantly reduced peribronchiolar extracellular matrix deposition, airway smooth muscle cell proliferation, and mucus production in the lung without affecting established airway inflammation and Th2 cytokine production. Thus, our data suggest that it might be possible to uncouple airway inflammation and remodeling during prolonged allergen challenge. In addition, anti-TGF-β Ab treatment was shown to regulate active TGF-β signaling in situ with a reduction in the expression of phospho-Smad 2 and the concomitant up-regulation of Smad 7 in lung sections. Therefore, this is the first report to suggest that anti-TGF-β Ab treatment prevents the progression of airway remodeling following allergen challenge even when given in a therapeutic mode. Moreover, the molecular mechanism behind this effect may involve regulation of active TGF-β signaling. The Journal of Immunology, 2005, 174: 5774–5780.

W e have demonstrated that prolonged allergen chal-

lenge in mice leads to the development of airway re-

modeling, an important pathophysiological feature of

chronic asthma (1). This model is characterized by persistent lung tissue eosinophilia and Th2 cytokine production. Moreover, this model exhibits many characteristics of human chronic asthma, namely increased bronchial subepithelial deposition of extracellu-

lar matrix, increased mucus production, and airway smooth muscle (ASM) cell proliferation (1–3). All of these features were found in association with an increase in lung tissue expression of the profibrotic mediator TGF-β1.

It has been hypothesized that TGF-β plays an important role in promoting the structural changes of tissue remodeling. In asthmas, increased TGF-β mRNA expression in bronchial biopsy sec-
tions was seen in comparison to normal subjects, and levels correlated with the depth of subepithelial fibrosis (4–7). In addition, bronchoalveolar lavage (BAL) levels of TGF-β1 were increased in asthmatic patients (8). TGF-β has been shown to be essential for the transformation of fibroblasts into myofibroblasts, which are major producers of collagen. Indeed, adenoviral-mediated gene transfer of TGF-β1 in the rodent lung, induced severe lung fibrosis with extracellular matrix deposition (9, 10). Hallmarks of the activ-

ation of the TGF-β signal transduction pathways are the phos-

phorylation of TGF-β type 1 (activin receptor-like kinase-5; ALK-5) and type II receptors and the subsequent phosphorylation and translocation of the intracellular effectors Smad 2 and Smad 3 (11) to the nucleus where they regulate gene transcription (12). Smad 7 is an intracellular inhibitor, which is rapidly induced by TGF-β family members and provides a negative feedback loop (13). Recent studies in a mouse model of acute allergic inflammation have demonstrated in situ activation of TGF-β signaling path-

ways in the airways (14, 15). However, the effect of therapeutic administration of anti-TGF-β Ab on the development of airway remodeling during prolonged allergen challenge in vivo has not yet been investigated.

We have previously shown that prolonged allergen challenge induced an increase in lung tissue levels of active TGF-β1 and cell-associated TGF-β1 production (1). The aim of this study was to determine the effects of blockade of TGF-β on the development of allergen-induced airway remodeling to identify a novel target for the treatment of airway remodeling during chronic asthma. Importantly, we administered a neutralizing Ab to TGF-β during the chronic phase of allergen-induced airway inflammation, after eosinophilic inflammation was established. We have demonstrated that treatment with TGF-β Ab modulated the development of all-

ergen-induced airway remodeling but had no effect on established inflammation. Therefore, our data indicate that it is possible to disengage inflammation from remodeling during prolonged aller-
gen challenge. Moreover, we report for the first time that anti-

TGF-β Ab treatment alters the progression of airway remodeling following allergen challenge via a molecular mechanism involving in situ regulation of active TGF-β signaling.

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3 Abbreviations used in this paper: ASM, airway smooth muscle; MSB, Martius Scarlet Blue; PCNA, proliferating cell nuclear Ag; BAL, bronchoalveolar lavage; pSmad, phosphorylated Smad; PAS, periodic acid-Schiff.
Materials and Methods

Allergen-induced airway inflammation and remodeling

Airway inflammation and remodeling was induced in female BALB/c mice sensitized with OVA in alum as previously described (Fig. 1, Ref. 1). Briefly, mice were sensitized using OVA (Sigma-Aldrich) at a concentration of 0.01 mg/mouse in 0.2 ml of alum (Au-Gel-S; Serva Electrophoresis) i.p. on days 0 and 12. Control mice received the same volume of PBS in alum (alum controls). All groups of mice were then challenged daily with 5% OVA (aerosolized for 20 min) via the airways between days 18 and 23. Prolonged inflammation was induced by subsequent exposure to aerosolized OVA (5%) three times a week for 20 min from day 26 onwards. We have previously shown that this prolonged protocol results in dramatic peribronchiolar extracellular matrix deposition and ASM cell proliferation as well as persistent mucus production, AHR, inflammation, and Th2 cytokine production, and is termed the chronic phase for the purposes of this study (1). A therapeutic treatment regimen was instigated by initiating treatment on day 26, after the establishment of considerable lung eosinophilia (Fig. 1). A neutralizing Ab to all mouse TGF-β isoforms was administered i.p. at a dose of 0.5 mg/kg (a gift from Cambridge Ab Technology) 30 min before each airway challenge. This dose has previously shown to be the optimum for a response in vivo (Ref. 16; M. McCourt, unpublished observation, Cambridge Antibody Technology). Control mice received the same dose of control Ig. In addition, another group of mice received PBS i.p. to show that administration of Ig had no effect on any parameters tested. Mice were then sacrificed on day 35 (after 4 doses) or on day 55 (after 13 doses).

Assessment of pulmonary inflammation

Cell recovery from airway lumen. BAL was performed as described (17). Briefly, mice were exsanguinated by cardiac puncture at sacrifice and the airway fluid collected. BAL fluid was centrifuged (700 × g, 5 min, 4°C); cells were counted then pelleted onto glass slides by cytocentrifugation (5 × 10⁴ cells/slide). Different cell types were performed on Giemsa (Shandon)-stained cytospins. Percentages of eosinophils, lymphocytes/monocytes, neutrophils and macrophages were determined by counting their number in eight high power fields (40 magnification; total area 0.5 mm² per area) and then dividing this number by the total number of cells obtained in the lavage fluid. All differential counts were performed blind and in a randomized order at the end of the study by the same observer.

Lung tissue histopathology. Paraflin-embedded sections (4 μm) were cut from anti-TGF-β- and control treated mice and fixed in 2% paraformaldehyde (Sigma-Aldrich) for 20 min. Endogenous peroxidase activity was blocked by incubation with 1.5% H₂O₂ in methanol for 30 min. Thereafter, endogenous avidin and biotin were blocked using a blocking kit (Vector Laboratories). All incubations were conducted in humidified chambers and slides were washed between steps for 15 min each in PBS containing 0.1% saponin (Sigma-Aldrich). Nonspecific binding was blocked using 10% donkey serum in PBS for 20 min. Sections were subsequently incubated with the primary Abs for 90 min. Polyclonal rabbit Abs against phosphorylated Smad 2 (pSmad 2), Smad 7, and the TGF-β type I receptor, also known as ALK-5, were raised and used as previously described (14). Normal rabbit IgG (Sigma-Aldrich) was used as a negative control. Sections were then incubated with biotinylated goat anti-rabbit secondary Ab (Stratagene) for 30 min, followed by streptavidin complex (DakoCytomation) for another 30 min. Slides were developed for ~5 min in DAB (Vector Laboratories) and counterstained in Gill’s hematoxylin. Sections were subsequently dehydrated, mounted, and studied by light microscopy.

Cytokine analysis

Cytokines were analyzed in lung tissue homogenates. Briefly, 100 μg of lung tissue from isolated lung lobes were homogenized in 2 ml of HBSS, centrifuged (800 × g, 10 min), and the resulting supernatant was assayed. Paired Abs for murine IL-5 (Perbio) were used in a standardized sandwich ELISA according to the manufacturer’s protocol. Kits to measure IL-13 were purchased from R&D Systems.

Statistical analysis

Results were expressed as mean ± SEM with a group size of 4–17 from four different experiments. Data was analyzed by ANOVA (Kruskal-Wallis) or Mann-Whitney U test where appropriate and statistical significance was accepted when p < 0.05.
Results
Administration of anti-TGF-β Ab decreased peribronchiolar extracellular matrix deposition during prolonged allergen challenge

Prolonged allergen challenge of sensitized mice results in increased extracellular matrix deposition in bronchiolar subepithelial regions in comparison to alum control mice (Fig. 2A; Ref. 1). We determined the effect of therapeutic administration of anti-TGF-β Ab on the extent of extracellular matrix deposition in lung tissue histologically, by staining lung sections with MSB. Administration of Ig control had no effect on the development of subepithelial extracellular matrix deposition in comparison to PBS-treated mice (Fig. 2B). In contrast, subepithelial extracellular matrix deposition was substantially reduced in lungs from mice treated with a neutralizing Ab to TGF-β in comparison to Ig-treated mice (Fig. 2A). To quantitate these differences in matrix deposition we performed image analysis to determine the relative density of matrix in 20-μm lengths of the basement membrane in lung sections from each group of mice. Fig. 2B shows that treatment with anti-TGF-β Ab significantly reduced peribronchiolar matrix density in the subepithelial region of mice at both time points during prolonged allergen challenge in comparison to Ig-treated mice.

FIGURE 2. Therapeutic administration of anti-TGF-β Ab decreased peribronchiolar extracellular matrix deposition. A, Representative photomicrographs of paraffin-embedded (4 μm) MSB-stained lung sections from mice treated with Ig control (left panel) or anti-TGF-β Ab (right panel) on day 55 are shown (original magnification ×20 and ×40). B, Image analysis of MSB-stained lung sections from mice treated with anti-TGF-β Ab, Ig, or PBS controls during prolonged allergen challenge. Random measurements from the basement membrane into the submucosa (10 measurements of 20 μm in length) were taken and the mean density was calculated from four bronchioles per mouse. Data is expressed as mean ± SEM; n = 6–8 mice per group. *, p < 0.05 in comparison to Ig-treated mice.

Administration of anti-TGF-β Ab decreased ASM-like cell proliferation

An increase in ASM mass is a characteristic of chronic asthma (24). Our model of prolonged allergen challenge is associated with an increase in numbers of total and proliferating ASM-like cells (as shown by positive PCNA staining in elongated and round nuclei cells) in the smooth muscle layer during the chronic phase (day 55) in comparison to alum controls (Refs. 1 and 25; Fig. 3). Administration of Ig control had no effect on the allergen-induced increase in ASM-like cell numbers or proliferation in comparison to PBS-treated mice (Fig. 3, B and C). Treatment with anti-TGF-β Ab significantly reduced numbers of total (Fig. 3, A and B) and proliferating (Fig. 3, A and C) ASM-like cells in comparison to Ig control mice on day 55 during prolonged allergen challenge.

Blockade of TGF-β reduced established mucus production

Excessive mucus secretion from hyperplastic goblet cells is a characteristic feature of chronic asthma. Indeed, persistence of mucous-producing goblet cells was observed during our model of prolonged allergen challenge in comparison to alum control mice (Fig. 4A, Ref. 1). Mucus secreting cells were quantified in PAS-stained sections from mice treated with either anti-TGF-β Ab (Fig. 4A) or Ig control (Fig. 4A) or PBS control mice (photomicrograph not shown). Administration of Ig control had no effect on the allergen-induced increase in PAS-positive cells in comparison to PBS-treated mice (Fig. 4B). Therapeutic administration of anti-TGF-β Ab reduced the number of mucus-producing goblet cells within the respiratory epithelium on day 35 and 55 (p < 0.05) during prolonged allergen challenge in comparison to Ig-treated mice (Fig. 4B).

FIGURE 3. Therapeutic administration of anti-TGF-β Ab decreased ASM-like cell proliferation during prolonged allergen challenge. A, Representative photomicrographs of total and proliferating (PCNA-positive cells stained brown) ASM-like cells in peribronchiolar regions after immunostaining with PCNA from mice treated with anti-TGF-β Ab (Fig. 4A) or Ig control (Fig. 4A) or PBS control mouse (photomicrograph not shown). Administration of Ig control had no effect on the allergen-induced increase in PAS-positive cells in comparison to PBS-treated mice (Fig. 4B). Therapeutic administration of anti-TGF-β Ab reduced the number of mucus-producing goblet cells within the respiratory epithelium on day 35 and 55 (p < 0.05) during prolonged allergen challenge in comparison to Ig-treated mice (Fig. 4B).
**FIGURE 4.** Administration of anti-TGF-β Ab decreased established mucus production from goblet cells. A. Representative photomicrographs of PAS-stained lung sections from OVA-challenged mice treated with either Ig control (left panel) or anti-TGF-β Ab (right panel) on day 55 (original magnification ×40). B. Lung tissue sections from mice treated with anti-TGF-β Ab or Ig-treated or PBS control mice during prolonged allergen challenge were stained with PAS and assigned a mucus score based on the percentage of staining according to Materials and Methods. Data is expressed as mean ± SEM; n = 6–8 per group. *, p < 0.05 in comparison to Ig control mice.

Administration of anti-TGF-β Ab had no effect on established airway inflammation

**Airway lumen.** Inflammatory cell recruitment to the airways is thought to be a major factor in the development of allergen-induced lung pathophysiology. We determined leukocyte subsets in the airway lumen by differential counts of BAL cytospins prepared from each treatment group. Administration of Ig control had no effect on the development of airway inflammation in comparison to PBS-treated mice at either time point (Fig. 5A). Therapeutic administration of anti-TGF-β Ab did not significantly affect numbers of any leukocyte subset in comparison to Ig-treated mice at either time point studied during prolonged allergen challenge (Fig. 5A).

**Lung tissue.** We determined the extent of leukocyte recruitment to the lung tissue by examination of H&E-stained histological sections. Fig. 5B (left panel) shows widespread peribroncholar inflammation in mice on day 55 of our chronic allergen challenge protocol. A similar degree of infiltration was observed in lungs from mice given a therapeutic regimen with anti-TGF-β Ab (Fig. 5B, right panel; histology score 3.93 ± 0.07) as compared with Ig control Ab-treated mice (Fig. 5B, left panel; histology score 3.64 ± 0.34).

**FIGURE 5.** Administration of anti-TGF-β Ab had no effect on established airway inflammation. A. Differential cell counts in BAL from mice treated with anti-TGF-β Ab, Ig control, or PBS on days 35 and 55 during prolonged allergen challenge. Mice were sacrificed 24 h after the final OVA challenge, and BAL cells were isolated as described in Materials and Methods. Values are expressed as mean ± SEM; n = 6–8 per group. B. Representative photomicrographs of H&E-stained lung sections from OVA-challenged mice treated with either Ig control (left panel) or anti-TGF-β Ab (right panel) on day 55 (original magnification ×20 and ×40).

Effect of administration of anti-TGF-β Ab on inflammatory mediator production

To gain some insight as to how treatment with anti-TGF-β Ab modulates matrix deposition, we next determined the concentration of the Th2 cytokines IL-5 and IL-13 in lung tissue homogenate supernatants. Production of IL-5 but not IL-13 persisted in the lungs of mice during prolonged allergen challenge in comparison to alum control mice (Fig. 6, A and B, Ref. 1). Administration of Ig control had no effect on lung tissue levels of the Th2 cytokines measured in comparison to PBS-treated mice (Fig. 6, A and B). Administration of anti-TGF-β Ab had no effect on levels of IL-5 or IL-13 in comparison to Ig-treated mice at either time point studied during prolonged allergen challenge (Fig. 6, A and B).

Anti-TGF-β Ab treatment modulated TGF-β signaling pathways

Because anti-TGF-β Ab had no effect on Th2 cytokine production, we next investigated the expression patterns of pSmad 2, ALK-5, and Smad 7 in lung tissues following anti-TGF-β Ab administration to delineate the possible mechanisms involved in TGF-β regulation. An increase in expression of pSmad 2 was observed during prolonged allergen challenge. Strong expression of pSmad 2 was detected in bronchial epithelial cells, vascular endothelial cells, inflammatory infiltrates, smooth muscle cells and alveolar macrophages in allergen-treated mice (Fig. 7A). Administration of anti-TGF-β Ab considerably decreased pSmad 2 expression on day 55 during prolonged allergen challenge (Fig. 7B). Only a few bronchiolar epithelial cells and alveolar macrophages were positive whereas none of the infiltrating leukocytes or smooth muscle cells expressed pSmad 2. ALK-5 was readily expressed in ASM cells together with bronchial epithelial and inflammatory cells in control-treated mice during prolonged allergen challenge (Fig. 7C). Administration of anti-TGF-β Ab abrogated the expression of ALK-5 in the ASM and decreased expression in all other cell populations on day 55 (Fig. 7D). Only weak Smad 7 immunostaining was observed in some bronchiolar epithelial cells and alveolar
macrophages in control-treated mice on day 35 during prolonged allergen challenge (Fig. 7E). Interestingly, marked up-regulation of Smad7 expression was detected in bronchiolar epithelium, infiltrating leukocytes, and alveolar macrophages upon administration of anti-TGF-β Ab (Fig. 7F).

Discussion

TGF-β is a potent fibrotic growth factor, and we postulated that blockade of TGF-β might affect the development of airway remodeling because pulmonary expression of TGF-β1 is up-regulated following prolonged allergen challenge (1). Administration of anti-TGF-β Ab after established airway inflammation reduced airway remodeling at both time points during prolonged allergen challenge as documented by a decrease in the mean density of peribronchiolar subepithelial matrix deposition. The anti-remodeling effect of anti-TGF-β Ab was more pronounced on day 35 in comparison to day 55. This is presumably due to less collagen deposition being observed and thus may be easier to block the development of airway remodeling at this early time point. Alternatively, at later stages of our model, other profibrotic cytokines such as platelet derived growth factor, connective tissue growth factor, epidermal growth factor as well as IL-11 may make a contribution, because these have all been implicated in the pathogenesis remodeling in asthma (26–28). Previous studies have demonstrated that direct administration of recombinant TGF-β1 protein to the airways of mice induced airway matrix deposition associated with mRNA expression of collagen I and III (29). Administration of anti-TGF-β1 and β2 Abs also reduced collagen deposition in the lungs of mice with bleomycin induced lung fibrosis (30). However, we have shown for the first time that therapeutic administration of neutralizing anti-TGF-β Ab during a prolonged allergen challenge model reduces the development of airway remodeling.

Mucus in the respiratory tract protects the lower airways from dehydration and damage; however, excessive secretion from hyperplastic goblet cells is thought to contribute to the morbidity and mortality of chronic asthma (31). We have previously shown that prolonged allergen challenge is associated with an increase in mucus-producing goblet cells in the bronchiolar epithelium (1). Our data demonstrate that treatment with anti-TGF-β Ab was associated with a significant decrease in mucus scores during prolonged allergen challenge. In support of our findings, TGF-β2 has been shown to induce bronchial epithelial mucin expression in the airways of asthmatics (32). This is the first report showing a beneficial effect of TGF-β blockade on airway mucus production in vivo.

Airway inflammation is a characteristic feature of allergic airway disease and infiltrating inflammatory cells to the airways are thought to contribute to the remodeling response. Indeed, we have recently shown that eosinophils are critical for the development of airway remodeling because mice with a total ablation of the eosinophil lineage were significantly protected from peribronchiolar collagen deposition and increases in ASM (25). Eosinophils express the profibrotic growth factor TGF-β (4, 6), thus, one of the potential roles of the eosinophil in asthma might include release of TGF-β and induction of airway remodeling. Interestingly, we demonstrated that administration of anti-TGF-β Ab during this model of prolonged allergen challenge had no significant effect on cell recruitment to the lung or on airway hyperreactivity (data not shown). Moreover, we found no change in levels of Th2 cytokines. In particular, levels of IL-13 were unchanged. This is of interest because it has been shown that IL-13 can induce lung fibrosis via activation of TGF-β (33). However, it has also been demonstrated that IL-13 induced fibrosis can occur independently of TGF-β (34). Although, many studies have shown that TGF-β is an important anti- and proinflammatory cytokine (35–37), this is the first report demonstrating therapeutic blockade of TGF-β during chronic allergic pulmonary inflammation. It is possible that TGF-β plays an important role in the development of acute inflammation in early stages post allergen challenge possibly induced by high levels of IL-13 (1, 33). Alternatively, because some regulatory
cells are known to secrete TGF-β, it might function in a regulatory capacity during the development of allergic inflammation (38, 39). We have shown that therapeutic treatment with TGF-β during prolonged allergen challenge reduced airway remodeling but had no effect on established airway inflammation. It is possible that activation of inflammatory cells during the initial phase of OVA challenge, i.e., before anti-TGF-β treatment, induced release of TGF-β. Under these circumstances, subsequent administration of the anti-TGF-β Ab could serve to attenuate the progressive development of airway remodeling without affecting inflammation. Alternatively, our findings may suggest that airway inflammation and remodeling can be uncoupled during prolonged allergen challenge, and might not necessarily be dependent on each other. Current thinking suggests that tissue remodeling occurs as a consequence of prolonged inflammation (2). However, evidence is emerging that remodeling may also occur via reactivation of the epithelial-mesenchymal trophic unit, an important component of the embryological development of the airways. This idea proposes that damage to the epithelium leads to activation of the attenuated myofibroblast sheath leading to the induction of further myofibroblasts and deposition ECM proteins (40). Consequently, some of the changes of airway remodeling may occur independently of inflammation. Although this theory has yet to be investigated fully in vivo, our data suggests that TGF-β released and/or acting directly upon structural cells plays an important role in the development and progression of airway remodeling. In vitro evidence might support this theory because TGF-β has been demonstrated to induce myofibroblast formation (41) and inflammatory chemokine production by lung fibroblasts (42). In our study, we have demonstrated that therapeutic administration of anti-TGF-β Ab decreased numbers of total and proliferating ASM cells coinciding with the decrease in extracellular matrix deposition. TGF-β is known to directly affect matrix production in resident lung cells, eliciting the secretion of collagen as well as glycosaminoglycans such as hyaluron from ASM cells in vitro (43, 44). Thus, our results indicate that TGF-β might also act directly in regulating extracellular matrix production from lung resident cells in vivo.

Recent reports have demonstrated an improved method for investigating the expression of active TGF-β signaling in situ which involved examination of the expression of the intracellular effectors, Smads (14, 15). We have shown active TGF-β signaling in the lung tissue of mice during prolonged allergen challenge in a model that exhibits many features similar to those of chronic asthma. In this respect, our data demonstrated that expression of the TGF-β type I receptor (ALK-5) and the intracellular effector phospho-Smad 2 is dramatically increased during prolonged allergen challenge in a number of different cell types including bronchial epithelial and endothelial cells, inflammatory leukocytes and in ASM cells. This expression occurs concomitantly with increased extracellular matrix deposition, mucus production and ASM cell proliferation. In contrast, we observed very little expression of the negative regulator Smad 7 in the lung tissue of OVA-treated mice during prolonged challenge as compared with alum control mice. Clinical studies using biopsies from asthmatic patients have also demonstrated a correlation between basement membrane thickness and activation of TGF-β signaling through an increased expression of Smad 2 in asthmatic airways (45). Moreover, Smad 7 was shown to be inversely correlated with basement membrane thickness in asthmatic airways (46). Interestingly, administration of neutralizing anti-TGF-β Ab regulated active TGF-β signaling in the airways as shown by a decrease in pSmad 2 and ALK-5 expression concomitant with de novo induction of the inhibitory molecule Smad 7. Our results suggest that treatment with anti-TGF-β Ab reduced collagen deposition via direct modulation of active TGF-β signaling. In support of this, TGF-β was recently shown to induce in vitro differentiation of myofibroblasts and collagen production through a Smad 2 dependent mechanism (47).

In conclusion, we have shown that TGF-β blockade decreased the development of airway remodeling in a model of prolonged allergen challenge. Moreover, our data suggest that airway inflammation and remodeling can be uncoupled during the development of chronic allergen-induced airway inflammation in this model because administration of anti-TGF-β Ab reduced the development of airway remodeling but not inflammation. Moreover, we have shown that the anti-remodeling effect of the anti-TGF-β Ab involved regulation of the TGF-β signaling pathway. Thus TGF-β might represent a novel target for the treatment of chronic asthma.

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Disclosures

The authors have no financial conflict of interest.

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