Induced Asthma – Cockroach Allergen

Mesenchymal Stem Cell Mobilization in

Functional Effects of TGF-β1 on

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Mesenchymal stem cells (MSCs) have been suggested to participate in immune regulation and airway repair/remodeling. TGF-β1 is critical in the recruitment of stem/progenitor cells for tissue repair, remodeling, and cell differentiation. In this study, we sought to investigate the role of TGF-β1 in MSC migration in allergic asthma. We examined nestin expression (a marker for MSCs) and TGF-β1 signaling activation in airways in cockroach allergen extract (CRE)–induced mouse models. Compared with control mice, there were increased nestin + cells in airways and higher levels of active TGF-β1 in serum and p-Smad2/3 expression in lungs of CRE-treated mice. Increased activation of TGF-β1 signaling was also found in CRE-treated MSCs. We then assessed MSC migration induced by conditioned medium from CRE-challenged human epithelium in air/liquid interface culture in Transwell assays. MSC migration was stimulated by epithelial-conditioned medium, but was significantly inhibited by either TGF-β1–neutralizing Ab or TβRI inhibitor. Intriguingly, increased migration of MSCs from blood and bone marrow to the airway was also observed after systemic injection of GFP + MSCs and from bone marrow of Nes-GFP mice following CRE challenge. Furthermore, TGF-β1–neutralizing Ab inhibited the CRE-induced MSC recruitment, but promoted airway inflammation. Finally, we investigated the role of MSCs in modulating CRE-induced T cell response and found that MSCs significantly inhibited CRE-induced inflammatory cytokine secretion (IL-4, IL-13, IL-17, and IFN-γ) by CD4 + T cells. These results suggest that TGF-β1 may be a key promigratory factor in recruiting MSCs to the airways in mouse models of asthma. The Journal of Immunology, 2014, 192: 4560–4570.
cells at sites of airway damage. It has been suggested that the mobilization of stem cells from bone marrow/blood circulation is regulated by locally released chemotactic substances (32). Indeed, our recent studies have suggested that activated TGF-β1 released from the injured vessels controls mobilization and recruitment of MSCs to participate in tissue repair/remodeling (33).

TGF-β1 is a multifunctional cytokine that plays a critical role in cell growth, differentiation, and immune regulation, and it has been considered a principal mediator of airway remodeling (34–38). TGF-β1, while in a latent form maintained in a sequestered state in the cell matrix, is considered to be a molecular sensor that releases active TGF-β1 in response to the perturbations of the extracellular matrix at the sites of mechanical stress, wound repair, tissue injury, and inflammation (39, 40). Recent studies have demonstrated that disruption in TGF-β1 signaling imposes a strong predisposition for human allergic diseases (41). Specifically, increased active TGF-β1 has been observed in airways from asthmatic patients (42) and from experimental mice during allergic airway inflammation (43). Furthermore, TGF-β1 has been shown to promote immune responses in the presence of MSCs (44). The stage is thus set to critically evaluate the functional significance of TGF-β1 signaling in the migration of MSCs, which may be critical in anti-inflammatory responses and tissue repair/remodeling.

In the present study, we have specifically focused on the functional significance of TGF-β1 activation in the migration of MSCs, which may be critical in modulating allergen-induced airway allergic inflammation. We found increased MSC expression and activated TGF-β1 signaling in airways of cockroach allergen extract (CRE)-induced mouse asthmatic models and in CRE-treated MSCs. We then demonstrated that active TGF-β1 released from CRE-challenged human epithelium is a primary allergen-activated messenger for MSC migration in vitro. Importantly, we observed the increased recruitment of MSCs to the airway from blood and bone marrow in the CRE-induced mouse asthmatic model in a TGF-β1-dependent process. Finally, we investigated the effect of TGF-β1 on allergen-induced allergic inflammation and the role of MSCs in modulating CRE-induced T cell responses. These studies provide an important basis for further detailed investigation of the role of TGF-β1 signaling in MSC-involved airway inflammation and repairing/remodeling in allergen-induced asthma.

**Materials and Methods**

**Mice**

Four- to 6-wk-old male and female C57BL/6J mice and nestin-GFP-transgenic mice (45) and C57BL/6J-GFP mice (46) were used. All animals were maintained under specific pathogen-free conditions in the animal facility of the Johns Hopkins University School of Medicine. The experimental protocols were reviewed and approved by the Animal Care and Use Committee at Johns Hopkins University School of Medicine.

**Cockroach allergen–induced asthma mouse model**

To generate a mouse model of cockroach allergen–induced asthma, mice were sensitized i.p. with cockroach extract (CRE) at a concentration of 20 μg/mouse with 2 mg aluminum hydroxide (alum) as adjuvant on days 0 and 7 and then challenged with the same amount of CRE via intranasal instillation for 3 successive days (days 21–23). Control mice received the same volume of PBS in alum. On day 24, mice were sacrificed, bronchovascular lavage fluid (BALF) was collected, and lungs were harvested for proposed studies. Serum was harvested for the measurement of cockroach allergen–specific IgE.

**Serum cockroach allergen–specific IgE measurement**

Serum IgE was depleted with protein G (GE Healthcare Life Sciences) to increase the sensitivity of IgE measurement according to Lehrer et al. (47). The IgF-depleted serum was analyzed by ELISA according to our previous work (48). Briefly, plates were coated with CRE (10 μg/ml) at 4°C overnight. Plates were blocked for 1 h with ELISA blocking buffer (eBioscience, San Diego, CA). Serum samples was added and incubated overnight at 4°C. Biotinylated anti-mouse IgE (BioLegend) was added, followed by streptavidin-HRP and tetramethylbenzidine substrate. Absorption at 450 nm was measured with a Bio-Rad iMark microplate absorbance reader.

**Lung histology**

Harvested lungs were fixed in 10% neutral buffered formalin overnight and embedded in paraffin or OCT for frozen section. Five-micrometer lung sections were cut and then stained with H&E to evaluate general morphology. For immunohistochemical staining, non-specific binding was blocked using 10% blocking serum in PBS for 1 h, and the tissue samples were then incubated with primary Abs to nestin (clone 10C2, Abcam), GFP (clone 4B10, Cell Signaling Technology), mannos-binding protein (MBP; clone MT-14.7, Lee Laboratory, Mayo Clinic), and p-Smad2/3 (Santa Cruz Biotechnology) overnight at 4°C. An HRP-streptavidin detection system (Dako, Glostrup, Denmark) was used to detect immunofluorescence activity followed by counterstaining with hematoxylin. For immunofluorescent staining, secondary Abs conjugated with fluorescence were added, and slides were incubated at room temperature for 1 h. Isotype-matched negative control Abs (R&D Systems) were used under the same

![FIGURE 1](http://www.jimmunol.org/)

**FIGURE 1.** Nestin* cells in lungs of CRE-challenged mice. (A and B) Representative H&E-stained sections from mice immunized and challenged with saline (A) and CRE (B). Original magnification ×20. (C) Dense peribronchial infiltrates. Score was defined by the number of infiltrates. (D) Serum levels of cockroach allergen–specific IgE. (E and H) Nestin* cells (MSCs, arrows) in airway sections of CRE- (E) and saline-treated mice (G) by nestin staining. Control IgG staining in airway sections of CRE- (F) and saline-treated mouse (H). (E–H) Original magnification ×60. **p < 0.01. Data are representative of three independent experiments (n = 4–6 mice/group).
conditions. Nuclei were counterstained with DAPI (Sigma-Aldrich, St. Louis, MO). The sections were mounted with the ProLong Gold anti-fade kit (Molecular Probes, Grand Island, NY) and observed under a microscope (Olympus). All histomorphometric parameters in four randomly selected visual fields per specimen and in five specimens per mouse in each group were measured. A similar approach was used for the immunofluorescent staining of p-Smad2/3 in cultured bone marrow–derived MSCs.

**BALF and cellular differential count**

Harvested BALF was centrifuged; the supernatants were then collected for the measurement of active TGF-β1 by ELISA (eBioscience). Total cell number was counted using a hemacytometer (Hauser Scientific), and cellular differential percentage was determined by means of flow cytometry. Briefly, BALF cells were first blocked with 10 μg/ml IgG Fc receptor blocking reagent (2.4G2, BD Biosystems) for 10 min, then stained for 30 min with the mixture of the following Abs: anti–CD107b (Mac-3)-FITC (M3/84, BD Biosystems), anti–Siglec-F-PE (E-50-2440, BD Biosystems), anti–Ly-6G (Gr-1)-PerCP-Cyanine5.5 (RB6-8C5, eBioscience), anti–CD3ε-allophycocyanin (145-2C11, eBioscience), and anti–CD19-allophycocyanin (1D3, eBioscience). Cells were analyzed on a FACSCalibur cytometer (BD Biosystems). Lymphocytes were identified as forward scatter<low>/side scatter<low> and expressing CD3 or CD19. Granulocytes were recognized as side scatter<high> Gr-1+ cells; eosinophils were defined as side scatter<high>Siglec-F+; and alveolar macrophages cells were identified as side scatter<high>Siglec-F+Mac-3+ cells.

**Western blotting**

Cells were washed twice with ice-cold PBS and lysed in RIPA buffer containing protease and phosphatase inhibitor cocktails (Sigma-Aldrich). Protein content was measured with BCA reagent (Pierce). Equivalent protein samples were subjected to SDS-PAGE electrophoresis and then transferred to a polyvinylidene difluoride membrane (Millipore). After blocking with 5% nonfat dry milk in TBST, the membrane was incubated with primary anti–p-Smad2/3 and anti-Smad2 (Santa Cruz Biotechnology). Proteins reactive with primary Abs were visualized with an HRP-conjugated secondary Ab and ECL reagents (Amersham).

**Migration assay**

To prepare the airway epithelial conditioned medium (ECM), we used an air/liquid interface (ALI) culture system for human airway epithelial cells. The isolated epithelial cells from central airway of human subjects (provided by Dr. Allen Myers, n = 3) were cultured for 21 d as described previously (49), then cultured with serum-free DMEM, and treated with CRE (B46, 100 μg/ml, Greer Laboratories) at 37˚C for 24 h; the cultured medium was collected and stored at −80˚C. Cell migration was performed in 96-well Transwells (Corning, Acton, MA). A total of 2 × 10^4 MSCs...
(Texas A&M, Institute for Regenerative Medicine) in 50 μl serum-free DMEM were placed in the precoated upper chambers with 0.5 μg/ml type I collagen (BD Biosciences, San Diego, CA), and 150 μl undiluted ECM was added to the type I collagen–coated lower chamber of the Transwell.

In some experiments, neutralizing Abs against TGF-β1 (1D11, R&D Systems), SDF1 (Cell Signaling Technology), or TGF-β type I receptor (TβRI) inhibitor (SB505124, Sigma-Aldrich) were added to the ECM. After 10 h of incubation, cells that migrated to the lower side of the filter were fixed with 10% formaldehyde, stained with hematoxylin (Sigma-Aldrich), and then counted under a microscope. Counted cells were expressed as number of migrated cells in five fields (×20).

**Isolation of MSCs**

Bone marrow–derived MSCs were isolated from mice and characterized using the established approaches in our previous work (50–52). In brief, the isolated cells from bone marrow were cultured with DMEM and 20% FBS (Atlanta Biologicals) at 37°C in a 5% CO2 humidified incubator. After 72 h, nonadherent cells were removed and adherent cells were cultured for an additional 14 d. The adherent cells were retrieved by 0.25% trypsin digestion containing 0.02% ethylenediaminetetraacetic acid and sorted by markers Sca-1+, CD29+, CD45−, and CD11b− (BioLegend). The sorted cells were enriched by further culture. A similar procedure was performed on GFP-labeled MSCs to sort GFP-labeled Sca-1+CD45−CD11b−MSCs (52).

**In vivo MSC migration by GFP+MSC injection or in Nes-GFP mice**

To examine the MSC migration from blood to the lung, sorted GFP+MSCs (2 × 10^6) from C57BL/6J-GFP mice were injected into cockroach allergen–sensitized C57BL/6J mice through the tail vein right before challenge (day 20). After a 3-d consecutive CRE challenge, mice were sacrificed on day 24, and lung tissues were harvested for the analysis of GFP+ cells. To further examine the migration of endogenous MSCs, we used Nes-GFP mice; a transgenic mouse reporter line expressing GFP under the control of enhancer/promoter of nestin gene (53), to generate CRE-induced mouse models of asthma using the same protocol as above. Similarly, lung tissues were harvested for the analysis of GFP+ cells. To see the effect of TGF-β1 on MSC migration, the cockroach allergen–sensitized mice were injected i.p. with TGF-β1 neutralizing Ab (1D11) or isotype control Ab (13C4) at a concentration of 0.25 mg/mouse on day 20 (1 d before the initial challenge). The mice were sacrificed at 24 h after the last challenge (day 24). Recruitment of MSCs to the allergen-challenged airways was analyzed by immunofluorescent staining for GFP.

**Flow cytometric analysis**

For the analysis of regulatory T cells (Tregs) in lung lymph nodes, the pulmonary hilar lymph nodes were collected and teased apart into a single-cell suspension by pressing with the plunger of a 3 ml syringe. The cells were stained with anti-CD4-FITC (RM4-5, eBioscience) and anti-CD25-PE Abs (PC61.5, eBioscience), followed by intracellular staining with Foxp3-allophycocyanin (FJK-16s, eBioscience) or allophycocyanin-conjugated rat IgG2a isotype control (eBioscience) using a Foxp3 staining kit (eBioscience). The samples were then analyzed on a FACSCalibur flow cytometer (BD Biosystems). Similar approaches were used for the analysis of TβRI and TβRII (Santa Cruz Biotechnology) in bone marrow–derived MSCs.

**Functional effect of MSCs on CRE-induced T cell responses**

To test whether MSCs could inhibit CRE-induced T cell response in vitro, mice were sensitized with 50 μg CRE plus 2 mg alum as adjuvant i.p. on days 0 and 7 and sacrificed on day 21. CD4+ T cells were purified from the spleen and lymph nodes using purification systems from Miltenyi Biotec.
TGF-β1 mediates MSC migration induced by human ECM

To further examine whether active TGF-β1 released from epithelium in response to environmental allergens can induce MSC migration, we performed a human ECM-based cell migration assay (Fig. 3A) in which ECM collected by incubating human epithelium with or without CRE challenge was placed in the bottom chamber of a Transwell and MSCs were placed in the upper chamber of the Transwell. Significantly greater numbers of migrated MSCs were observed in the group with ECM prepared from allergen-challenged epithelial cells, compared with those from the resting cells (Fig. 3B, 3C). To examine whether TGF-β1 is one of the major factors in CRE-challenged ECM responsible for the increased migration of MSCs, we first measured the levels of active TGF-β1 in ECM. A significantly greater amount of active TGF-β1 was observed in ECM prepared by CRE-challenged epithelial cells when compared with those from the resting cells (Fig. 3D). To further examine whether TGF-β1 in ECM is essential for MSC migration, we added TGF-β1-neutralizing Abs in ECM for the migration assay. Interestingly, the migration of MSCs was almost abolished when TGF-β1-neutralizing Ab was used. The migration of MSCs was not affected by adding Ab against SDF-1α/CXCL12, a chemoattractant that can induce the migration of many cell types (Fig. 3E) (55). When different doses of TβRI kinase-specific inhibitor SB50512 (0.5, 1.0, and 2.0 μM) were added to ECM for the migration assay, the ECM-induced migration was significantly inhibited in a concentration-dependent manner (Fig. 3F), suggesting that TGF-β1 signaling is a primary pathway that drives MSC migration. To validate the direct effect of TGF-β1 on MSC migration, we analyzed the migration of MSCs by the addition of the recombinant TGF-β1 in different doses into the DMEM in the lower chamber of the Transwell. Significant MSC migration was seen at 5, 10, and 20 ng/ml TGF-β1 in a dose-dependent manner (Fig. 3G, 3H).

TGF-β1 mediates the recruitment of MSCs to the lungs in asthma

Although MSCs were increased in airway after CRE sensitization and challenge, it remains unclear whether these MSCs are bone marrow derived or local in origin. We thus examined whether there

**Results**

**MSCs are accumulated in lung tissue of CRE-challenged mice**

MSCs have been shown to be increased in the lungs after allergen sensitization and challenge (13, 21–23). To investigate whether cockroach allergen can also induce MSC migration to the lungs, we established a cockroach allergen–induced asthmatic mouse model. Compared with control mice (Fig. 1A), cockroach allergen–induced models showed significant recruitment of inflammatory cells to the lung (Fig. 1B) and dense peribronchial infiltrates (Fig. 1C) in the histological examination as well as increased serum levels of cockroach allergen–specific IgE (Fig. 1D). We then used this established model to examine whether MSCs migrated to the airway after cockroach allergen sensitization and challenge. It was reported that nestin can serve as a marker for bone marrow MSCs that have both self-renewal and multilineage potential in vivo (54). Our histological analysis demonstrated a high number of nestin+ cells in airway epithelial cells and subepithelial inflammatory cells from mice after CRE challenge (Fig. 1E), as compared with saline-treated mice (Fig. 1F). No positive staining was seen for control IgG (Fig. 1F, 1H). The results suggest that MSCs are increased in airway after allergen sensitization and challenge.

**TGF-β1 signaling is activated in lung tissue of allergic asthma and in CRE-treated MSCs**

Our recent studies have suggested that activated TGF-β1 released from the injured vessels controls mobilization and recruitment of MSCs to participate in tissue repair/remodeling (33). To investigate whether TGF-β1 signaling is involved in the migration of MSCs to lungs in asthma, we examined the levels of active TGF-β1 in blood and BALF in CRE-challenged mice. The concentrations of active TGF-β1 were significantly higher in both BALF (p < 0.01, Fig. 2A) and peripheral blood (p < 0.01, Fig. 2B) in mice after allergen challenge, when compared with control mice. We also detected p-Smad2/3 in the airways of the mice. As compared with those of saline-treated mice (Fig. 2C), much higher numbers of p-Smad2/3+ epithelial cells and subepithelial inflammatory cells were found in the airways of mice after CRE challenge (Fig. 2D). The results indicate that cockroach allergen induces the activation of TGF-β1 signaling in airways in asthma. To further examine whether cockroach allergen can induce the activation of TGF-β1 signaling in MSCs, we detected the expression of TGF-β receptors (TβRI, TβRII), cockroach allergen–induced TGF-β1 secretion, as well as p-Smad2/3 expression in MSCs. We found that both TβRI (Fig. 2E) and TβRII (Fig. 2F) were constitutively expressed in MSCs, and MSCs can secrete a large amount of active TGF-β1 in response to cockroach allergen (Fig. 2G). Furthermore, cockroach allergen can induce the increased activation of TGF-β1 signaling in MSCs as determined by Western blotting (Fig. 2H) and immunofluorescent staining (Fig. 2I).

**Statistical analysis**

Data are expressed as the means ± SEM for each group. Statistical significance for normally distributed samples was assessed using an independent two-tailed Student t test or with ANOVA by using GraphPad Prism version 5.1 software (GraphPad Software, La Jolla, CA). Differences with p < 0.05 were considered statistically significant.
was an increased recruitment of MSCs to the lung from peripheral blood or bone marrow. We first examined whether transplanted MSCs can be recruited to the lungs in CRE-treated mice. These transplanted MSCs were sorted by markers Sca-1+, CD29+, CD45−, and CD11b− (Fig. 4A). The ability of MSCs to differentiate into fibroblasts/myofibroblasts was evaluated by the expression of α-smooth muscle actin (Abcam) with DAPI for nuclei immunostaining after cells were treated with CRE (50 μg/ml) for 72 h (Fig. 4B). A total of 2 × 10^6 sorted MSCs were then injected into CRE-challenged mice through the tail vein right before CRE challenge according to the protocol in Fig. 5A. Significantly greater numbers of GFP+ cells were observed in the lungs from these CRE-sensitized and challenged mice, when compared with those in the lungs that received saline alone (Fig. 5B, 5C). To examine whether TGF-β1 is required for GFP+ MSC recruitment, we systemically injected TGF-β1-neutralizing Ab on the day before MSC injection and CRE challenge. We found that the increased GFP+ MSCs in lungs of CRE-induced mice were significantly inhibited when the mice were pretreated with TGF-β1-neutralizing Ab (Fig. 5B, 5C). Next, we examined whether TGF-β1 is also essential for the recruitment of

**FIGURE 5.** MSCs mobilize to the lungs from peripheral blood through TGF-β1. (A) Schematic of experimental protocol for mouse models of asthma. (B) Immunofluorescence analysis of injected GFP+ MSCs in the airways of CRE-challenged or saline-treated mice with or without TGF-β1 Ab. Original magnification ×20. (C) Number of injected GFP+ MSCs was counted per field of view (original magnification ×20) and analyzed. Bars represent means ± SEM for four to six mice per group. (D) GFP+ cells in the airways of CRE-challenged or saline-treated Nes-GFP mice. Original magnification ×40. (E) Total numbers of MSCs in BAL detected by flow cytometry (anti-GFP). Bars represent means ± SEM of three independent experiments. *p < 0.05, **p < 0.01.
endogenous MSCs to the lungs in asthma using Nes-GFP mice, in which MSCs express GFP under the regulatory elements of the nestin promoter (54). Increased numbers of engrafted GFP+ MSCs in the airways (Fig. 5D) and in BALF (Fig. 5E) were observed after the mice were treated with CRE. No nestin+ cells were detected in other tissues, including liver, kidney, spleen, heart, adipose tissue, and aorta, after allergen sensitization and challenge (data not shown). Importantly, the increased GFP+ MSCs were significantly diminished in mice receiving TGF-β1–neutralizing Ab (Fig. 5D, 5E). These findings suggest that TGF-β1 is a key factor that recruits MSCs from bone marrow/peripheral blood to the airway following CRE sensitization and challenge.

**TGF-β1 limits allergic inflammation**

We then examined whether TGF-β1 can suppress cockroach allergen–induced allergic inflammation in our established cockroach allergen–induced mouse models. Increased recruitment of total inflammatory cells (Fig. 6A) and neutrophils, eosinophils, macrophages, and lymphocytes (Fig. 6B) were detected in BALF of the mice following CRE treatment. Moreover, dense peribronchial infiltrates were also found in the lung tissue by histological examination (Fig. 6C). TGF-β1 Ab treatment further elevated total numbers of inflammatory cells in BALF, specifically eosinophils (Fig. 6B), and increased the airway inflammation (Fig. 6C) in CRE-induced mice. The increase of eosinophils in the airway was further confirmed by staining for MBP, a marker for eosinophils (Fig. 6D). The increased inflammation in lungs after treatment with TGF-β1–neutralizing Ab was correlated with decreased numbers of MSCs detected in the airway (Fig. 5D) and in BALF (Fig. 5E). Additionally, TGF-β1 has been shown to be essential for Treg development that is capable of suppressing inflammation in vivo (56). To test whether TGF-β1 suppresses inflammation through Tregs, we examined the percentage of CD4+CD25+Foxp3+ Tregs in the lung hilar lymph nodes in TGF-β1–neutralizing Ab-treated and untreated mice. As shown in Fig. 6E–G, no significant difference was observed in the percentage of Tregs between TGF-β1–neutralizing Ab-treated and untreated groups. The results suggest that the effect of TGF-β1 on inflammatory response is not through a direct effect on Treg development.

**MSCs modulate T responses to CRE in vitro**

Bone marrow–derived MSCs have been shown to suppress allergic responses in a mouse model of ragweed-induced asthma (57), but the mechanism remains unclear. It is possible that TGF-β1–neutralizing Ab may increase the immune response through inhibiting MSC recruitment. To test this possibility, we performed an in vitro analysis to examine whether MSCs can inhibit CRE-induced T cell responses. Indeed, when we cocultured the CD4+ T cells isolated from CRE-sensitized mice with irradiated spleen cells and treated the cells with CRE for 3 d, production of IL-4 (Fig. 7A), IL-13 (Fig. 7B), IL-17 (Fig. 7C), and IFN-γ (Fig. 7D) was significantly increased. However, when MSCs were added to the cocultures, these cytokines were dramatically inhibited.

**Discussion**

In the present study, we evaluated the functional significance of TGF-β1 activation in the recruitment of MSCs to the airways in asthma. We examined whether MSCs can be recruited to the lungs after cockroach allergen sensitization and challenge, and, most importantly, identified the primary promigratory factors that control the MSC migration in this process. We found increased MSCs and TGF-β1 activation in lungs of cockroach allergen–induced mouse models and an increased activation of TGF-β1 signaling in CRE-treated MSCs. Furthermore, using an ECM-based Transwell assay, we found that active TGF-β1 released from allergen-activated epithelium is a primary allergen-activated messenger for MSC migration. Findings from these studies were further validated by the detection of increased numbers of transplanted GFP+ MSCs and endogenous MSCs (GFP+ cells) in the lung tissues in mouse asthma models. Importantly, the neutralizing Ab against TGF-β1 abrogated the migration of MSCs in both ex vivo ECM-based migration assay or in vivo cockroach allergen–induced
mouse models. Thus, TGF-β1 is a primary promigratory factor produced in the CRE-challenged lung tissue to control the recruitment of MSCs in asthma.

MSCs have the ability of self-renewal and differentiation into multiple cell types in vitro and in vivo (12). Several lines of evidence have suggested the involvement of MSCs in asthma (13, 21–23). Our studies have provided further supporting evidence that MSCs are significantly increased after cockroach allergen sensitization and challenge. Histological analysis demonstrated a high number of nestin+ MSCs in airway epithelial and subepithelial layers from mice after CRE challenge as compared with saline. Notably, when we detected MSCs in the airway tissues from patients with allergic asthma, we found that those nestin+ MSCs were only found in the airway subepithelial region, not in airway epithelium (data not shown). It is likely that MSCs from patients with chronic asthma have resumed their mesenchymal features and are associated with fibrosis/remodeling in the subepithelial region. In contrast, MSCs from patients with acute asthma may be recruited into airway epithelial regions and undergo associated differentiation into lung epithelial cells (58).

Moreover, we have used Nes-GFP–transgenic mice to track the endogenous MSC migration to the lung tissues, and found that the recruitment of Nes-GFP+ cells was increased after cockroach allergen challenge as compared with saline. By contrast, when we detected MSCs in the airway tissues from patients with allergic asthma, we found that those nestin+ MSCs were only found in the airway subepithelial region, not in airway epithelium (data not shown). It is likely that MSCs from patients with chronic asthma have resumed their mesenchymal features and are associated with fibrosis/remodeling in the subepithelial region. In contrast, MSCs from patients with acute asthma may be recruited into airway epithelial regions and undergo associated differentiation into lung epithelial cells (58). Moreover, we have used Nes-GFP–transgenic mice to track the endogenous MSC migration to the lung tissues, and found that the recruitment of Nes-GFP+ cells was increased after cockroach allergen sensitization and challenge. Nestin, a class VI intermediate filament protein, was originally described as a neuronal stem cell marker expressed during CNS development. Specifically, nestin is expressed mainly in migrating and proliferating cells during development, whereas in adult tissues nestin is mainly expressed in bone marrow MSCs and neuronal stem cells in brain. More than 95% of MSCs express nestin. However, postnatal nestin expression in neuronal stem cells is extremely limited unless there is brain or nerve injury (59, 60). Nestin+ bone marrow cells have also been functionally characterized as MSCs based on their CFU fibroblastic activity, ability to be propagated as nonadherent “mesenspheres” that can self-renew and expand in serial transplantations and in vivo contribution to osteochondral lineages under homeostasis (54). Whereas these Nes-GFP+ cells in our models mark the MSC population in bone marrow (61), we could not absolutely exclude the possibilities of the resident lung–derived MSCs or some types of airway epithelial cells types functioning as progenitors or stem cells that are positive for nestin. Future studies are needed on the clarification of whether bone marrow nestin+ MSCs are the main source of lung nestin+ cells after allergen challenge. We have also examined nestin+ cells in other tissues, including liver, kidney, spleen, heart, adipose tissue, and aorta, after allergen sensitization and challenge in cockroach allergen–induced Nes-GFP transgenic mouse models of asthma. We found that no nestin+ cells were detected in these examined other tissues after cockroach allergen sensitization and challenge, suggesting that the damaged lung tissue is the major target site where the nestin+ cells were recruited.

Active TGF-β1 has been observed to be increased in human asthmatic airways (42, 62), sputum supernatants (63), and airway in mouse models during allergic reactions (43). Overexpression of TGF-β1 in primary airway fibroblasts has been associated with the regulation of granulocyte activation and trafficking (63) and progression of airway remodeling (64). Our studies provided further evidence for the increased activation of TGF-β1 signaling in mouse models of asthma by showing increased levels of active TGF-β1 in BALF and peripheral blood, as well as p-Smad2/3+ in airway of mouse models during allergic reactions. Although active TGF-β1 can be released from different cellular sources, such as bronchial epithelium (65), eosinophils (66), macrophages (67), and fibroblasts (64), our study suggests that MSCs may also be one of the major cell types releasing active TGF-β1 and have an increased activation of TGF-β1 signaling in response to cockroach

FIGURE 7. Modulatory effects of MSCs on CRE-induced T responses in vitro. MSCs were cocultured with isolated CD4+ T cells from CRE-sensitized mice. Cytokines including IL-4 (A), IL-13 (B), IL-17 (C), and IFN-γ (D) were measured by ELISA. Bars represent means ± SEM of three independent experiments. **p < 0.01. Med, medium.
allergen treatment. In this study, we found that MSCs constitutively express both TβRI and TβRII and have increased p-Smad2/3 when exposed to cockroach allergen.

We observed increased levels of active TGF-β1 released from cockroach allergen–treated primary epithelial cells under the ALI culture, which includes the isolation of airway epithelial cells from central airway, culturing of these isolated cells at ALI, and exposure to experimental allergen (49, 68). The ALI culture model allows the culture of airway epithelia in response to allergen exposure that more closely resembles their physiological setting than ordinary liquid culture system (69). We postulated that active TGF-β1 released from damaged airway epithelial cells after repeated exposure to allergens may be a major resource for promoting MSC recruitment to the lung tissues. Indeed, we found that the migration of MSCs was significantly inhibited when either TGF-β1–neutralizing Ab or TβRI inhibitor was added into ECM that was used for migration assays. Furthermore, we examined the role of TGF-β1 signaling in the recruitment of MSCs to the lung tissue in mouse asthma models. We found that TGF-β1–neutralizing Ab can significantly inhibit MSC migration from blood circulation to the lungs. Interestingly, the total number of inflammatory cells, especially eosinophils, was enhanced by TGF-β1–neutralizing Ab. Additionally, we found a high number of p-Smad2/3 cells in the lungs from mice after CRE challenge, and the increased p-Smad2/3 cells were abrogated by TGF-β1–neutralizing Ab. This was consistent with the previous report indicating that anti–TGF-β1 can regulate active TGF-β1 signaling in situ with a reduction of p-Smad2 in lung sections.

Very recent studies by Frischmeyer-Guerrerio et al. (41) demonstrated that disruption in TGF-β1 signaling imposes a strong predisposition for human allergic diseases, and they suggested that inhibition of TGF-β1 signaling could be a potential basis for beneficial therapeutic strategies. However, our studies demonstrated that TGF-β1 plays an anti-inflammatory role in an allergen-induced mouse model of asthma. In particular, the blocking of TGF-β1 can promote allergic inflammation as determined by increased total numbers of inflammatory cells in BALF and peribronchial infiltrates. In particular, significantly increased eosinophils were observed in BALF and lung tissue from anti–TGF-β1-treated mice. This is consistent with the previous report that TGF-β1 can suppress eosinophilic lung disease (70), and mutations in the receptor for TGF-β1 are associated with a strong predisposition toward eosinophilic gastrointestinal disease (41). In contrast, MSCs in BALF and lung tissue were significantly reduced, providing evidence for the possibility that the enhanced allergen-induced allergic inflammation by anti-TGF-β1 may be due to the reduction of migrated MSCs. Indeed, injected bone marrow–derived MSCs have been shown to suppress Th2-driven allergic responses in a mouse model of ragweed-induced asthma through TGF-β1 (57). However, the detailed underlying mechanism concerning the suppressive role of MSCs in inflammation remains unclear. To test the possibility that TGF-β1 suppressed inflammation through Tregs, we examined the percentage of CD4+CD25+Foxp3+ Tregs in the lung hilar lymph nodes from TGF-β1–neutralizing Ab–treated and untreated mice. However, no significant difference was observed for the percentage of Tregs, suggesting that TGF-β1–neutralizing Ab may not directly affect Treg development. Thus, it is likely that these migrated MSCs may be the major factor in suppressing inflammation by TGF-β1. In fact, MSCs have been suggested to be involved in modulating the immune responses through paracrine effects and interaction with different immuneocytes (26–30). In this study, we found that MSCs significantly inhibited CRE-induced inflammatory cytokine secretion by CD4+ T cells (IL-4, IL-13, IL-17, and IFN-γ). The findings provide further evidence for the recruited MSCs in modulating immune and inflammatory responses in asthma. However, we failed to find clear differences in the percentage of Th1 and Th2 cells among CD4+ T cells isolated from these lymph nodes of CRE-treated mice between TGF-β1–neutralizing Ab–treated and untreated mice (data not shown). This suggests the complexity of the underlying mechanisms regarding TGF-β1–suppressed inflammation.

The role of TGF-β1 in modulation of inflammation is controversial. The model presented in this study represents an acute type immunological response where the data clearly demonstrate a role of the recruited MSCs in modulating immune and inflammatory responses in asthma. We think, in the early stage, active TGF-β1 released from damaged/repairing epithelium in response to allergens suppresses immune response either directly by inhibiting the function of immune cells, such as T cells, B cells, NK cells, and macrophages, or indirectly by recruiting MSCs into the major damaged lung tissue to suppress inflammation by the secretion of cytokines and growth factors at sites of tissue inflammation and repair the damaged epithelium. While in the late stage, the sustained active TGF-β1 released locally owing to repetitive allergen challenge may cause aberrant excessive recruitment of MSCs and promote MSC differentiation into myofibroblasts. This will lead to a progressive fibrosis and pathological remodeling in asthma.

Taken together, our studies have demonstrated that TGF-β1 released from allergen-activated epithelium is a primary promigratory factor controlling the recruitment of MSCs to the lungs in asthma. Moreover, we demonstrated that MSCs can inhibit CRE-induced inflammatory cytokine secretion by CD4+ T cells. These findings suggest that TGF-β1 may play a role in MSC migration, which could be critical in allergen-induced airway allergic inflammation. Importantly, these studies provide a basis for a further investigation into the role of TGF-β1 in regulating MSC-involved airway inflammation and remodeling/remodeling in allergen-induced asthma.

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Disclosures

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