

Hepatocyte Growth Factor Attenuates Airway Hyperresponsiveness, Inflammation, and Remodeling

Wataru Ito, Arihiko Kanehiro, Kunio Matsumoto, Atsushi Hirano, Katsuichiro Ono, Hiromi Maruyama, Mikio Kataoka, Toshikazu Nakamura, Erwin W. Gelfand, and Mitsune Tanimoto

Second Department of Internal Medicine, Okayama University Medical School, Okayama; Biomedical Research Center, Osaka University Graduate School of Medicine, Osaka, Japan; and Program in Cell Biology, Department of Pediatrics, National Jewish Medical and Research Center, Denver, Colorado

Hepatocyte growth factor (HGF) is known to influence a number of cell types and their production of regulatory cytokines. We investigated the potential of recombinant HGF to regulate not only the development of allergic airway inflammation and airway hyperresponsiveness (AHR), but also airway remodeling in a murine model. Administration of exogenous HGF after sensitization but during ovalbumin challenge significantly prevented AHR, as well as eosinophil and lymphocyte accumulation in the airways; interleukin (IL)-4, IL-5, and IL-13 levels in bronchoalveolar lavage (BAL) fluid were also significantly reduced. Further, treatment with HGF significantly suppressed transforming growth factor- β (TGF- β), platelet-derived growth factor, and nerve growth factor levels in BAL fluid. The expression of TGF- β , the development of goblet cell hyperplasia and subepithelial collagenization, and the increases in contractile elements in the lung were also reduced by recombinant HGF. Neutralization of endogenous HGF resulted in increased AHR as well as the number of eosinophils, levels of Th2 cytokines (IL-4, IL-5, and IL-13) and TGF- β in BAL fluid. These data indicate that HGF may play an important role in the regulation of allergic airway inflammation, hyperresponsiveness, and remodeling.

Keywords: airway hyperresponsiveness; airway inflammation; airway remodeling; hepatocyte growth factor; transforming growth factor- β

Bronchial asthma is a syndrome associated with allergen-induced chronic airway inflammation and airway hyperresponsiveness (AHR). The pathophysiology of AHR is complex, and many factors contribute to its development. Airway mucosal inflammation is characterized by an influx of activated eosinophils and T lymphocytes (1), and numerous investigations have identified that Th2 cytokines (particularly interleukin [IL]-4, IL-5, and IL-13) play critical roles in orchestrating the allergic inflammatory response leading to AHR (2–6). Airway structural changes occur in response to persistent inflammation (airway remodeling), and include subepithelial fibrosis, hyperplasia of mucus glands, myofibroblast and smooth muscle proliferation, and vascular changes (7). Transforming growth factor- β (TGF- β), which accelerates fibrotic changes through the accumulation of extracellular matrix, may play a key role in this airway remodeling process; TGF- β expression correlates with basement membrane thickness and fibroblast number (7, 8). Furthermore, although TGF- β is reported to be an important factor in the regulation of acute

pulmonary inflammation, as in pneumonia (9), the role of growth factors such as TGF- β in asthma remains to be defined.

Hepatocyte growth factor (HGF) was originally identified and cloned as a potent mitogen for mature hepatocytes, and is now recognized as a potent stimulator of a variety of epithelial cell types (10, 11) and a humoral mediator of epithelial–mesenchymal interactions (11). Furthermore, HGF injections result in reduced expression of TGF- β in animal models (12–14). Recent studies have suggested that HGF prevents apoptosis during embryogenesis and organogenesis in mice targeted with the HGF gene (15, 16), and administration of human recombinant HGF prevented the onset and progression of hepatic fibrosis/cirrhosis, renal, lung, and myocardial fibrosis (12–14, 17–21).

To define the role of HGF in regulating the development of allergen-induced AHR, inflammation, and airway remodeling, we monitored lung function in response to inhaled methacholine (MCh), inflammatory cell infiltration in the airways, cytokine/growth factor levels in bronchoalveolar lavage (BAL) fluid, and changes in epithelial cell function following sensitization and challenge to ovalbumin (OVA). We demonstrate that HGF has significant regulatory effects on all of these parameters.

MATERIALS AND METHODS

Animals

Female BALB/c mice 8–10 wk of age were purchased from Charles River Japan, Inc. (Yokohama, Japan). Interferon (IFN)- γ -deficient mice (BALB/c background) were obtained from Jackson Laboratories (Bar Harbor, ME). The mice were maintained on diets free of OVA. All experimental animals used in this study were housed under constant temperature and light cycles, and under a protocol approved by the Institutional Animal Care and Use Committee of Okayama University Medical School.

Sensitization and Airway Challenge

Mice (4 mice/group/experiment) receiving the following treatments were studied: (1) nonsensitized and nonchallenged mice, and (2) OVA-sensitized and OVA airway-challenged mice (OVA/OVA). Mice were immunized by intraperitoneal injection of 20 μ g OVA (Grade V; Sigma, St. Louis, MO) emulsified in 2.25 mg alum (AlumImject; Pierce, Rockford, IL) in a total volume of 100 μ l on Days 0 and 14. Mice were challenged via the airways with OVA (1% in saline) for 20 min on Days 28, 29, and 30 by ultrasonic nebulization (Figure 1). AHR was assessed 48 h after the last challenge, and tissues and cells were obtained for further assays (22, 23).

In a repeated allergen exposure model, mice were further exposed to aerosolized 1% OVA for 20 min, 3 d/wk for two additional weeks (2 wk) (2 wk-OVA/OVA) or four additional weeks (4 wk) (4 wk-OVA/OVA) (Figure 1). AHR and immunologic examinations were assessed 48 h after the last challenge.

Administration of Human Recombinant HGF

Human recombinant HGF (hrHGF) was a gift from Dr. T. Nakamura (Osaka University School of Medicine, Osaka, Japan) (24). Mice received subcutaneous injections of hrHGF (125, 250, or 500 μ g/kg daily), from Days 27–31 in the short exposure model (Figure 1). In the repeated

(Received in original form February 13, 2004 and in final form November 1, 2004)

This work was supported in part by a grant from the Ministry of Education, Science and Culture of Japan, and was funded in part by grants from the National Institutes of Health HL-36577 and HL-61005, and the Environmental Protection Agency grant R825702.

Correspondence and requests for reprints should be addressed to Arihiko Kanehiro, M.D., Second Department of Internal Medicine, Okayama University Medical School, 2-5-1 Shikata-cho, Okayama 700-8558, Japan. E-mail: akanehir@md.okayama-u.ac.jp

Am J Respir Cell Mol Biol Vol 32, pp 268–280, 2005

Originally Published in Press as DOI: 10.1165/rcmb.2004-0058OC on December 30, 2004
Internet address: www.atsjournals.org

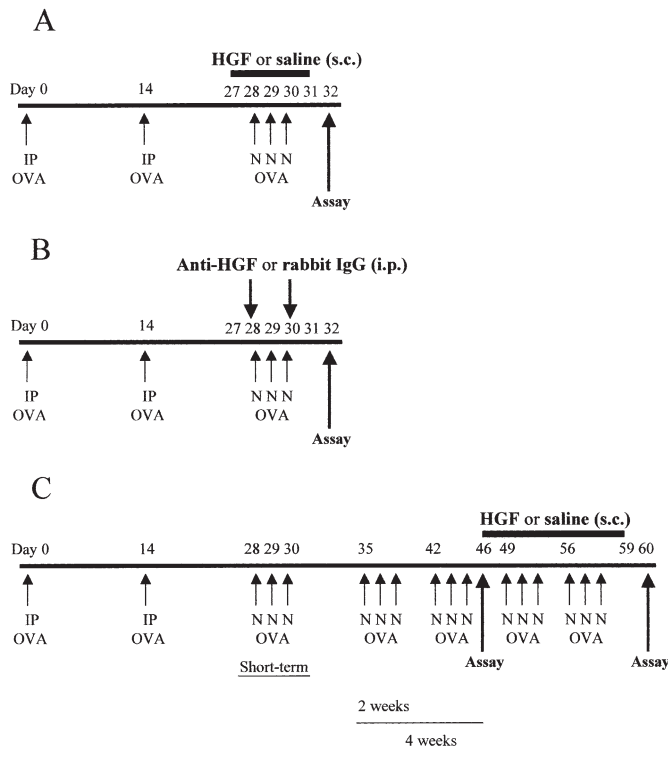


Figure 1. Experimental protocols. (A) Mice were sensitized by two intraperitoneal injections of OVA/alum and then received three consecutive days of aerosolized OVA challenge (short-term challenge). To evaluate the effect of HGF on AHR and airway inflammation, mice received subcutaneous injections of human recombinant HGF (125, 250, 500 $\mu\text{g/kg}$ daily) from Days 27–31. (B) To assess the effect of anti-HGF on AHR and airway inflammation, mice received intraperitoneal injections of the anti-rat HGF IgG (250 $\mu\text{g}/\text{mouse}$) on Days 28 and 30. (C) To define the effect of HGF on the airways after repeated OVA challenges, mice were exposed to aerosolized 1% OVA for 20 min on 3 d/wk for an additional 2 or 4 wk, and received subcutaneous injections of hrHGF (300 $\mu\text{g/kg}$ daily) from Days 46–59 for 2 wk.

exposure model, mice received subcutaneous injections of hrHGF (300 $\mu\text{g/kg}$ daily) from Days 46–59. As a control, mice were administered saline subcutaneously (Figure 1).

Anti-HGF Antibody Treatment

To inhibit HGF, anti-hepatocyte growth factor (anti-HGF) antibody was raised by immunizing normal rabbits with rat HGF (25, 26); the IgG fraction was purified using Protein A-Sepharose (Pharmacia Biotech, Uppsala, Sweden). This anti-rat HGF crossreacts with mouse HGF, but not with human HGF. The antibody inhibits branching tubulogenesis in fetal kidneys (25) as well as in lungs (26). One microgram of anti-rat HGF IgG neutralizes the biological activity of at least 5 ng of mouse HGF (27, 28). Mice received intraperitoneal injections of the anti-rat HGF IgG (250 $\mu\text{g}/\text{mouse}$) on Days 28 and 30. As a control, mice were administered normal rabbit IgG (250 $\mu\text{g}/\text{mouse}$) intraperitoneally (27) (Figure 1).

Determination of Airway Responsiveness

Airway responsiveness was assessed as a change in airway function after challenge with aerosolized MCh using barometric plethysmography (Buxco Electronics Inc., Troy, NY) as described (29). Pressure differences were measured between the main chamber of the plethysmograph, containing conscious, spontaneously breathing animals and the reference chamber (box pressure signal). Mice were challenged with aerosolized saline (for the baseline measurements) or MCh (1.56–25 mg/ml) for 3 min, and readings were taken and averaged for 3 min after each

nebulization. Data were expressed using the dimensionless parameter enhanced pause (Penh) as described (30).

BAL and Measurement of BAL Fluid Cytokines

After assessment of Penh, lungs were lavaged via the tracheal tube with saline (2×1 ml, 37°C). The volume of collected BAL fluid was measured in each sample and the number of BAL cells was counted. Cytospin slides were stained and differentiated in a blinded fashion by counting at least 300 cells under light microscopy. Cytokine concentrations in the BAL fluid supernatants were measured by ELISA. The colorimetric measurements were performed according to the manufacturer's instructions. The limits of detection were 4 pg/ml for IL-4, IL-5, IL-12, IL-13, IFN- γ , and platelet-derived growth factor (PDGF; R&D Systems, Minneapolis, MN). The limit of detection was 10 pg/ml for total TGF- β (Promega, Madison, WI) and nerve growth factor (NGF; Chemicon, Temecula, CA). The limit of detection for murine HGF was 0.4 ng/ml (Institute of Immunology, Tochigi, Japan).

Measurement of Serum Anti-OVA Antibody

Anti-OVA IgE antibody levels were measured by ELISA, 48 h after the last airway challenge as previously described (31). The antibody titers of samples were related to pooled standards that were generated in the laboratory and expressed as ELISA units per milliliter (EU/ml).

Histological and Immunohistochemistry Studies

After obtaining the BAL fluid, right lungs were inflated through the tracheal tube with 2 ml air and fixed in 10% formalin. Blocks of lung tissue were cut around the main bronchus and embedded in paraffin blocks. Tissue sections 4 μm thick were affixed to microscope slides and deparaffinized. The slides were stained with hematoxylin-eosin and periodic acid Schiff (PAS) for identification of mucus-containing cells (31), and were examined under light microscopy. In hematoxylin and eosin-stained lung sections, the numbers of total leukocytes and eosinophils per square millimeter in the peribronchial and perivascular tissue were analyzed using the NIH Image Analysis system (National Institutes of Health, Bethesda, MD). More than 10 bronchioles in a minimum of 10 high-power fields per lung tissue were randomly examined in a blinded fashion. The numbers of mucus-containing cells (goblet cells) were counted in more than 10 bronchioles in the 10 high-power fields per animal by measuring the length of epithelium defined along the basement membrane and luminal area using the NIH Image Analysis system.

Left lung tissues were fixed in ethanol for 12 h, dehydrated, and embedded in paraffin. Each section was cut to a thickness of 4 μm . To visualize expression of TGF- β , rabbit IgG against TGF- β (1:250; Promega) was applied to the dewaxed sections for the primary reactions, followed by an avidin-biotin coupling immunoperoxidase technique (13). To identify myofibroblasts, peroxidase-conjugated mouse monoclonal IgG against human α -smooth muscle actin (α -SMA; DAKO, Glostrup, Denmark) was used.

The area of Masson's trichrome-positive peribronchiolar collagen layer or α -SMA-positive peribronchiolar smooth muscle was measured using the NIH Image Analysis system (32, 33). All bronchioles of the size and shape defined by this system were selected, not only the bronchioles demonstrating airway collagen (34). The NIH Image Program allows for manual outlining of the trichrome-stained collagen layer or α -SMA-stained smooth muscle layer, and computes the area within the outlined ring of tissue. The perimeter is the airway basement membrane circumference. More than 10 bronchioles in a minimum of 10 high-power fields per lung tissue were randomly examined in a blinded fashion.

Statistical Analysis

All results were expressed as the mean $\pm$ SEM. ANOVA was used to determine the levels of difference among all groups. Pairs of groups were compared with unpaired two-tailed Student's *t* test or the Tukey-Kramer honest significant difference (HSD) test. The significance was set at $P < 0.05$.

RESULTS

Treatment with HGF Attenuates AHR and Inflammatory Cell Accumulation in BAL Fluid

To determine the effects of HGF on the development of altered airway function to inhaled MCh after OVA sensitization and challenge, mice received daily subcutaneous injections of human recombinant HGF or saline from Days 27–31, and AHR was assessed on Day 32. There were no significant differences in baseline Penh values among nonsensitized and nonchallenged mice (0.49 ± 0.04), OVA-sensitized and -challenged mice receiving saline (0.53 ± 0.17), and OVA-sensitized and -challenged mice receiving 500 $\mu\text{g/kg}$ HGF (0.53 ± 0.13). After OVA sensitization and challenge, AHR to inhaled MCh significantly increased in a dose-dependent manner compared with nonsensitized and nonchallenged mice. Administration of HGF to OVA-sensitized and -challenged mice prevented the increases in AHR throughout the MCh dose-response curve, in a dose-dependent manner (Figure 2A).

The numbers and types of inflammatory cells in the airways were determined in BAL fluid 48 h after the last of the three consecutive allergen challenges (Day 32). In nonsensitized and nonchallenged mice, very few inflammatory cells other than macrophages ($> 95\%$) were detected. Sensitization and challenge with OVA resulted in a marked increase in the number of eosinophils and lymphocytes in BAL fluid (Figure 2C). Administration of HGF daily, from 24 h before the first OVA challenge to 24 h after the last challenge, significantly reduced the number of eosinophils and lymphocytes as well as total cell numbers in BAL fluid in a dose-dependent manner. These findings indicate that administration of exogenous HGF can suppress both the development of AHR and airway inflammation.

Neutralization of Endogenous HGF Enhances AHR and Eosinophil Accumulation in BAL Fluid

Given the effects of HGF on AHR and inflammation, we examined the consequences of neutralizing endogenous HGF on these responses. Mice received intraperitoneal injections of anti-HGF IgG on Days 28 and 30, which resulted in neutralization of more than 80% of plasma HGF (data not shown). Administration of anti-HGF to OVA-sensitized and -challenged mice significantly enhanced AHR compared with OVA-sensitized and -challenged mice that received normal rabbit IgG (Figure 2B). Furthermore, HGF neutralization also significantly increased the number of total cells and eosinophils in BAL fluid compared with the control group (Figure 2D). Although not significant, there was a trend with increases in the number of lymphocytes in BAL fluid after treatment with anti-HGF compared with controls. In the lung, the number of lymphocytes was significantly increased after the administration of anti-HGF from $1,674.1 \pm 377.4$ to $2,297.4 \pm 355.1$ ($P < 0.05$). These findings suggest that neutralization of endogenous HGF results in enhanced AHR and airway inflammation, further supporting the important role of HGF in regulating these responses.

After treatment with anti-HGF, the number of PAS-positive cells was significantly increased in the OVA/OVA group from 197.8 ± 21.7 to 257.5 ± 27.3 ($P < 0.05$) compared with control IgG. Further, airway collagen deposition was significantly increased from 722.8 ± 172.3 to 867.4 ± 129.7 ($P < 0.05$), whereas smooth muscle hyperplasia was not significantly altered by anti-HGF administration (682.5 ± 162.6 to 706.1 ± 171.5).

Treatment with HGF Attenuates AHR and Inflammatory Cell Infiltrates in Chronically Exposed Mice

We assessed and compared AHR to inhaled MCh and the numbers and types of inflammatory cells in BAL fluid after different

challenge protocols, on Days 32, 46, and 60 (Figure 1). Maximum changes in AHR developed 48 h after the last of the initial three consecutive challenges (Day 32); however, AHR was still significantly increased even after 4 wk of repeated challenges (Day 60) when compared with controls (Figure 3A). Numbers of eosinophils in the BAL fluid peaked after the initial challenges but remained increased with repetitive challenges (Figure 3C). Lymphocyte numbers in repeatedly OVA-exposed mice were significantly increased over numbers in short-term challenged mice.

To determine whether HGF remained effective in modifying altered airway function and the inflammatory cell accumulation in mice after 4 wk of additional allergen exposure, mice received subcutaneous injections of hrHGF (250 $\mu\text{g/kg}$ daily) from Days 46–59 (Figure 1). Administration of exogenous HGF to repeatedly challenged mice significantly prevented the development of AHR (Figure 3B) and reduced number of eosinophils and lymphocytes in BAL fluid compared with the control groups (Figure 3D).

The effects of HGF on inflammatory cell infiltration were further investigated by histological examination of hematoxylin and eosin-stained slides (Figure 4). In nonsensitized and nonchallenged mice, very few eosinophils or lymphocytes were detected in the peribronchial and perivascular areas (Figure 4A). Sensitization and subsequent challenge with OVA via the airways increased the number of eosinophils and lymphocytes (Figures 4B and 4C). Examination of tissue sections showed that treatment with HGF significantly reduced both total inflammatory cell and eosinophil infiltration (Figures 4D and 4E) at these sites compared with saline-treated mice in both the short-term and repeated exposure models (Figures 4F and 4G). The number of lymphocytes in the lung was also decreased after the administration of HGF (OVA/OVA + saline; $1,674.1 \pm 377.4$ versus OVA/OVA + HGF; 673.9 ± 112.1 [$P < 0.05$]; 4 wk-OVA/OVA + saline; $2,431.6 \pm 472.2$ versus 4 wk-OVA/OVA + HGF; 977.4 ± 141.8 [$P < 0.05$]).

Treatment with HGF Inhibits Airway Remodeling after Chronic Allergen Exposure

Lung sections were stained with PAS to identify mucus-containing cells in the airway epithelium (Figure 5). A significant increase in numbers of PAS+ cells was found in OVA-sensitized and -challenged mice (Figure 5B) compared with nonsensitized and nonchallenged mice (Figure 5A). Numbers of cells staining positive for mucus increased with increased allergen exposure (Figure 5C), and treatment with HGF daily from Days 27–31 (Figure 5D) or from Days 46–59 (Figure 5E) significantly reduced the number of PAS+ cells per millimeter of basement membrane (Figure 5F).

The areas defined as the Masson's trichrome-positive peribronchiolar collagen layer (Figures 6A–6C) and the α -SMA-stained smooth muscle layer (Figures 6D–6F) were quantified using the NIH Image Analysis system. The area of Masson's trichrome-positive peribronchiolar collagen layer or α -SMA-positive peribronchiolar smooth muscle was measured using the NIH Image Analysis system (32, 33). Compared with nonsensitized and nonchallenged mice, collagen deposition/fibrosis and the thickness of the smooth muscle layer surrounding the airways were increased after increased allergen exposure. Treatment with exogenous HGF significantly inhibited airway collagen deposition (Figures 6C and 6G) and smooth muscle hyperplasia (Figures 6F and 6H).

Changes in HGF Levels after Repeated OVA Challenge

We examined the levels of HGF in BAL fluid during the course of OVA challenge of sensitized mice. The levels of HGF in

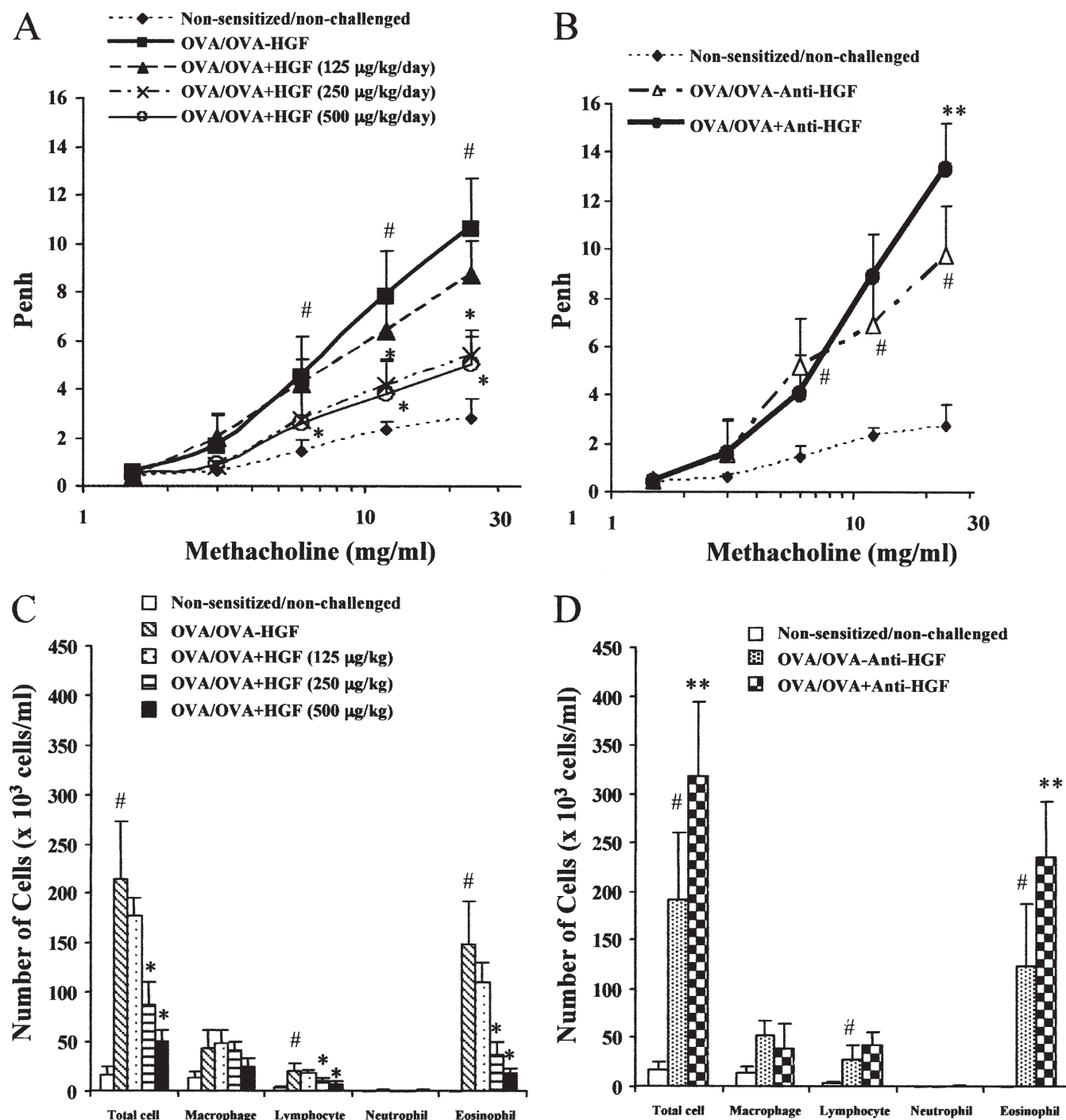


Figure 2. Treatment with HGF prevents the development of AHR and inflammatory cell accumulation in BAL fluid; anti-HGF effects on AHR and inflammatory cell accumulation. Penh values to increasing concentrations of inhaled MCh were measured as described in MATERIALS AND METHODS in nonsensitized/nonchallenged mice, OVA-sensitized/OVA-challenged mice receiving saline (OVA/OVA-HGF), and OVA-sensitized/OVA-challenged mice receiving HGF (OVA/OVA+HGF). Results for each group are expressed as the mean $\pm$ SD ($n = 16$ in each group). # Significant differences ($P < 0.05$) between nonsensitized/nonchallenged mice and OVA-sensitized/OVA-challenged mice. * Significant differences ($P < 0.05$) between OVA-sensitized/OVA-challenged control mice (receiving saline) (OVA/OVA-HGF) and HGF-treated mice (OVA/OVA+HGF). ** Significant differences ($P < 0.05$) between OVA-sensitized/OVA-challenged control mice (receiving IgG) (OVA/OVA-anti-HGF) and anti-HGF-treated mice (OVA/OVA+anti-HGF).

sensitized and challenged mice were significantly increased compared with nonsensitized and nonchallenged mice, and the highest HGF levels were observed in mice exposed to a further 2 wk of OVA challenges; levels were lower when challenges

continued for an additional 2 wk (4 wk) (Table 1). Administration of exogenous HGF to mice challenged for four additional weeks further increased HGF levels in BAL fluid, findings consistent with previous *in vitro* results that exogenous HGF increases

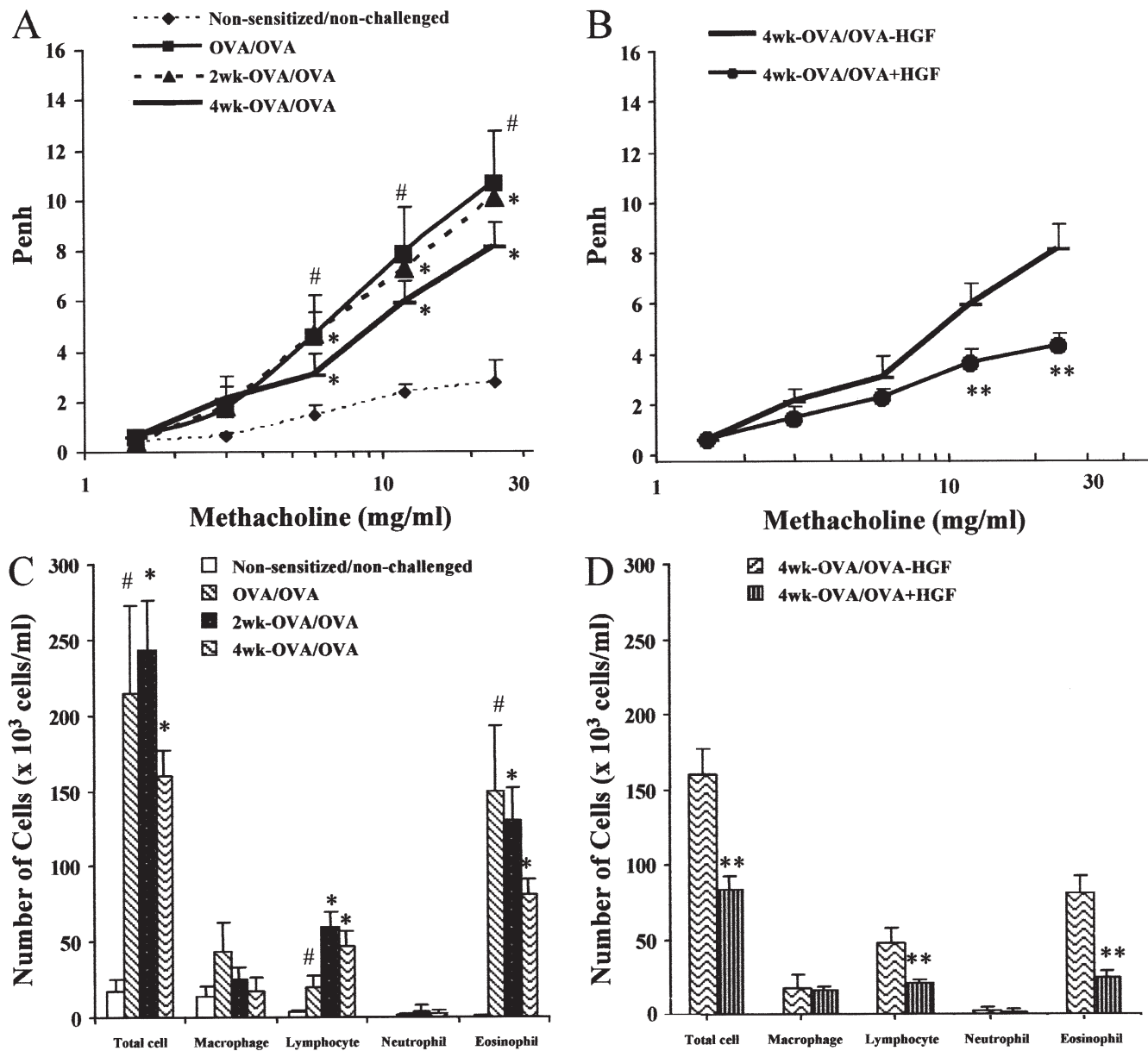


Figure 3. Treatment with HGF prevents AHR and inflammatory cell infiltration in mice subjected to repeated challenge with OVA. Data represent the mean $\pm$ SD ($n = 16$ in each group). # Significant differences ($P < 0.05$) between nonsensitized/nonchallenged mice and OVA-sensitized/OVA-challenged mice (OVA/OVA). * Significant differences ($P < 0.05$) between nonsensitized/nonchallenged mice and OVA-sensitized/OVA-chronic challenged mice (2 wk-OVA/OVA and 4 wk-OVA/OVA). ** Significant differences ($P < 0.05$) between OVA-sensitized/OVA-challenged mice (receiving saline) (4 wk-OVA/OVA-HGF) and HGF treated mice (4 wk-OVA/OVA+HGF).

endogenous HGF levels (35, 36). In sensitized and challenged mice that received anti-HGF, the levels of HGF were restored to baseline.

Treatment with HGF Inhibits Serum Anti-OVA IgE Antibody Levels

After OVA sensitization and challenge, serum anti-OVA IgE levels were significantly increased compared with nonsensitized and nonchallenged mice. Administration of exogenous HGF significantly reduced serum OVA-specific IgE levels compared with nontreated OVA-sensitized and -challenged mice (Table 2).

Treatment with Exogenous HGF Decreases and Neutralization of Endogenous HGF Increases Cytokine and Growth Factor Levels in BAL Fluid

Concentrations of cytokines (IL-4, IL-5, IL-13, IL-12, IFN- γ) and growth factors (PDGF, NGF, TGF- β) in BAL fluid were measured by ELISA. In sensitized and challenged mice, the levels of Th2 cytokines, IL-4, IL-5, and IL-13, and growth factors such as PDGF, NGF, and TGF- β in BAL fluid were significantly increased compared with nonsensitized and nonchallenged mice (Figure 7). In the BAL fluid, TGF- β levels were not different between the OVA/OVA group and 4 wk-OVA/OVA group. In

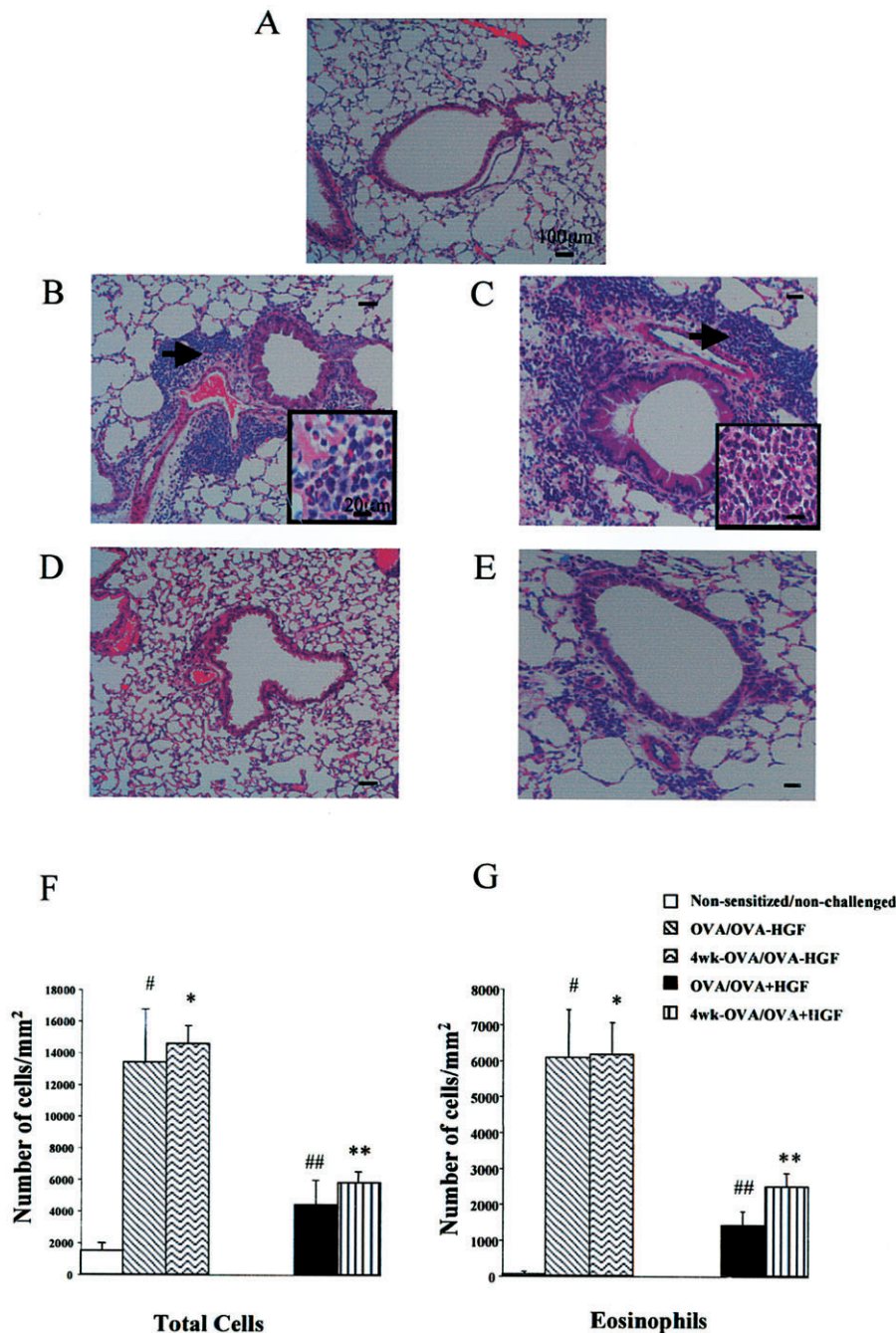


Figure 4. Effect of HGF on inflammatory cell accumulation in peribronchial and perivascular tissue. Evidence of inflammatory cell infiltration was investigated by histologic examination of hematoxylin-eosin-stained tissue as described in MATERIALS AND METHODS (final magnification: $\times 400$; inset: $\times 1,000$). (A) Nonsensitized and non-challenged, (B) OVA/OVA-HGF, (C) 4 wk-OVA/OVA-HGF, (D) OVA/OVA+HGF, (E) 4 wk-OVA/OVA+HGF. Total inflammatory cell (F) and eosinophil (G) numbers in the peribronchial and perivascular tissue. Data represent the mean $\pm$ SD ($n = 16$ in each group). # Significant differences ($P < 0.05$) between nonsensitized/nonchallenged mice and OVA-sensitized/OVA-challenged mice (OVA/OVA-HGF). * Significant differences ($P < 0.05$) between nonsensitized/nonchallenged mice and OVA sensitized and OVA chronic challenged mice (4 wk-OVA/OVA-HGF). ## Significant differences ($P < 0.05$) between OVA-sensitized/OVA-challenged mice (receiving saline) (OVA/OVA-HGF) and HGF-treated mice (OVA/OVA+HGF). ** Significant differences ($P < 0.05$) between OVA-sensitized/OVA-4 wk challenged mice (receiving saline) (4 wk-OVA/OVA-HGF) and HGF-treated mice (4 wk-OVA/OVA+HGF).

contrast, IL-12 levels were significantly decreased. Administration of exogenous HGF significantly reduced the levels of IL-4, IL-5, and IL-13 as well as PDGF, NGF, and TGF- β in BAL fluid compared with control mice (Figure 7). BAL fluid IL-12 levels in sensitized and short-term challenged HGF-treated mice were significantly increased, but HGF showed no significant effect in mice exposed to additional challenges (Figure 7E). TGF- β levels were modestly decreased with repeated challenges, whereas IFN- γ levels in the BAL fluid were unchanged in all of the study groups (Figure 7D).

Administration of anti-HGF to OVA-sensitized and short-term challenged mice resulted in significantly increased levels of Th2 cytokines (IL-4, IL-5, and IL-13), some increase in NGF, and a marked increase in TGF- β levels in BAL fluid compared

with mice receiving normal rabbit IgG; administration of anti-HGF had no effect on IL-12 or PDGF levels (Figure 7).

Localization of TGF- β in Lung Tissue

There is increasing support for the role of TGF- β in acute inflammatory disorders as well as in chronic inflammatory fibrotic disorders (37, 38). Significant increases in TGF- β staining were observed in sensitized and challenged mice (Figures 8B and 8G), whereas only slight positive immunolabeling for TGF- β was detected in the airway epithelium of nonsensitized and nonchallenged mice (Figures 8A and 8G). Positive immunolabeling for TGF- β was detected in eosinophils and mononuclear cells (data not shown) as well as airway epithelium in the peribronchial and perivascular areas in mice after OVA sensitization and short-term

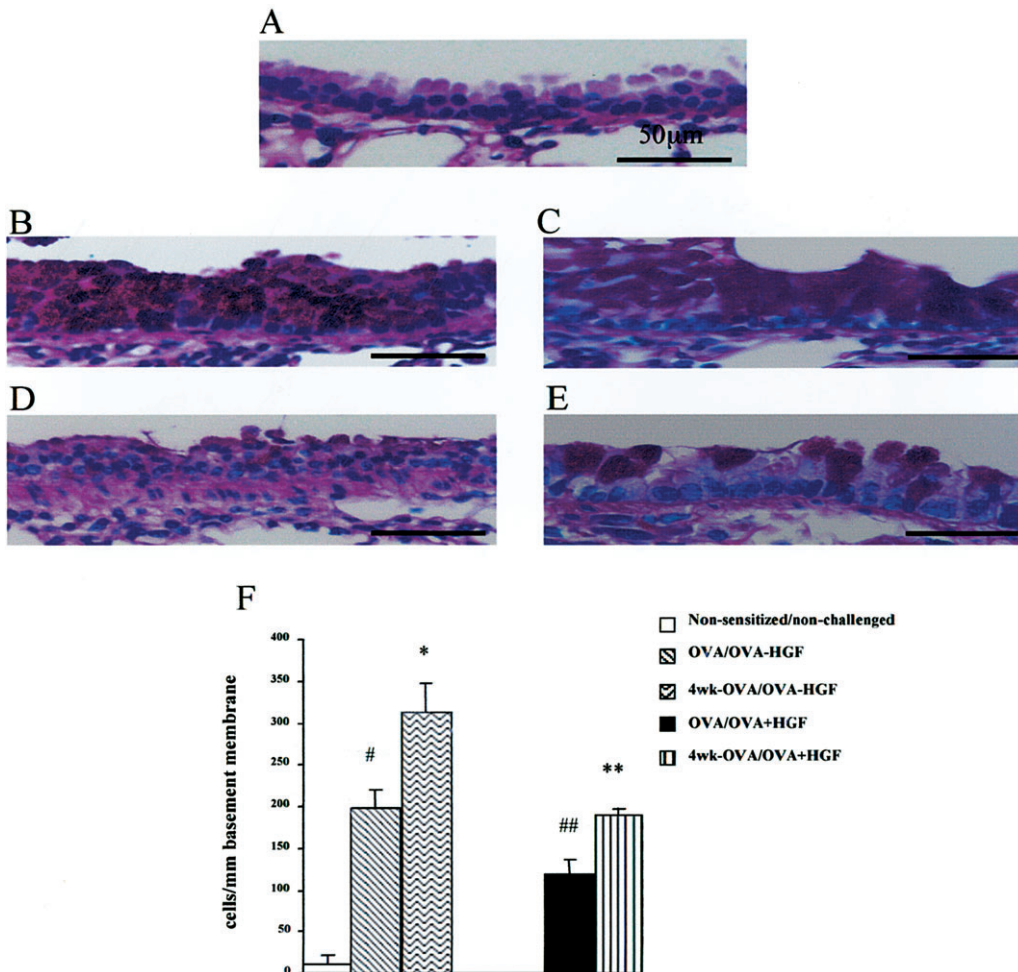


Figure 5. Treatment with HGF suppresses goblet cell hyperplasia in airway epithelium of OVA-sensitized and -challenged mice. PAS staining was performed to identify mucus-containing cells in the airway epithelium as described in MATERIALS AND METHODS (final magnification: $\times 1,000$). (A) Nonsensitized and nonchallenged, (B) OVA/OVAHGF, (C) 4 wk-OVA/OVAHGF, (D) OVA/OVA+HGF, (E) 4 wk-OVA/OVA+HGF. The number of mucus-positive cells per millimeter basement membrane. Data represent the mean $\pm$ SD ($n = 16$ in each group). # Significant differences ($P < 0.05$) between nonsensitized/nonchallenged mice and OVA-sensitized/OVA-challenged mice (OVA/OVA-HGF). * Significant differences ($P < 0.05$) between nonsensitized/nonchallenged mice and OVA-sensitized and OVA chronic challenged mice (4 wk-OVA/OVA-HGF). ## Significant differences ($P < 0.05$) between OVA-sensitized/OVA-challenged mice (receiving saline) (OVA/OVA-HGF) and HGF-treated mice (OVA/OVA+HGF). ** Significant differences ($P < 0.05$) between OVA-sensitized/OVA-4 wk challenged mice (receiving saline) (4 wk-OVA/OVA-HGF) and HGF-treated mice (4 wk-OVA/OVA+HGF).

(Figures 8B and 8G) and repeated challenge (Figures 8C and 8G). Treatment with exogenous HGF resulted in marked decreases in numbers of TGF- β positively stained cells and the extent of staining was lower compared with control mice (Figures 8D, 8E, and 8G). In contrast, administration of anti-HGF to OVA-sensitized and -challenged mice increased the numbers of TGF- β -stained cells (Figures 8F and 8G). These findings suggest that the balance between HGF and TGF- β may play a role in the development of airway inflammation and airway remodeling.

Effect of HGF in IFN- γ -Deficient Mice

As IFN- γ can regulate airway inflammation and airway responsiveness (39, 40) and IFN- γ may upregulate the HGF receptor (41), we determined whether the ability of HGF to negatively regulate AHR and airway inflammation is through induction of IFN- γ , using IFN- γ -deficient mice. The levels of endogenous HGF in OVA-sensitized and -challenged IFN- γ -deficient mice were 3.62 ± 1.94 . There were no significant differences compared with OVA-sensitized and -challenged BALB/c mice (3.80 ± 1.82). In contrast to wild-type mice, administration of exogenous HGF to OVA sensitized and challenged IFN- γ -deficient mice failed to influence development of AHR (Figure 9A) or the number of eosinophils in BAL fluid (Figure 9B). Furthermore, treatment with HGF in IFN- γ -deficient mice had no significant effect on IL-4, IL-5, and IL-13 levels in BAL fluid (Figures 9C–9E). There were no significant differences in IL-12 levels when IFN- γ -deficient mice were treated or not treated with

HGF (Figure 9F), implying that the regulatory mechanisms exhibited by HGF may be independent of IL-12. However, exogenous HGF did significantly reduce PDGF, NGF, and TGF- β levels in BAL fluid in IFN- γ -deficient mice compared with the control mice (Figures 9G–9I).

DISCUSSION

The pathophysiology of asthma is complex, with allergens triggering a cascade of cellular interactions and the release of cytokines and mediators in sensitized individuals, resulting in acute and delayed (late) symptoms. Th2 cells, through the release of specific cytokines and chemokines, regulate inflammatory cell recruitment to the lung, leading to AHR. In contrast to cytokines/chemokines, the function of growth factors in asthma has been less fully delineated and the results have not always been consistent. Minshall and coworkers demonstrated that TGF- β may play a role in the fibrotic changes occurring within asthmatic airways, and that activated eosinophils are a major source of this growth factor (42). Recently, Yamashita and coworkers demonstrated that PDGF was involved in the augmentation of airway responsiveness through remodeling of airways in diesel exhaust particulate-exposed mice (43). NGF, which is released from the airway epithelium and lung fibroblasts, is also reported to contribute to airway inflammation and AHR, via effects on inflammatory cells. NGF alone may also induce AHR in the absence of airway inflammation (44–46).

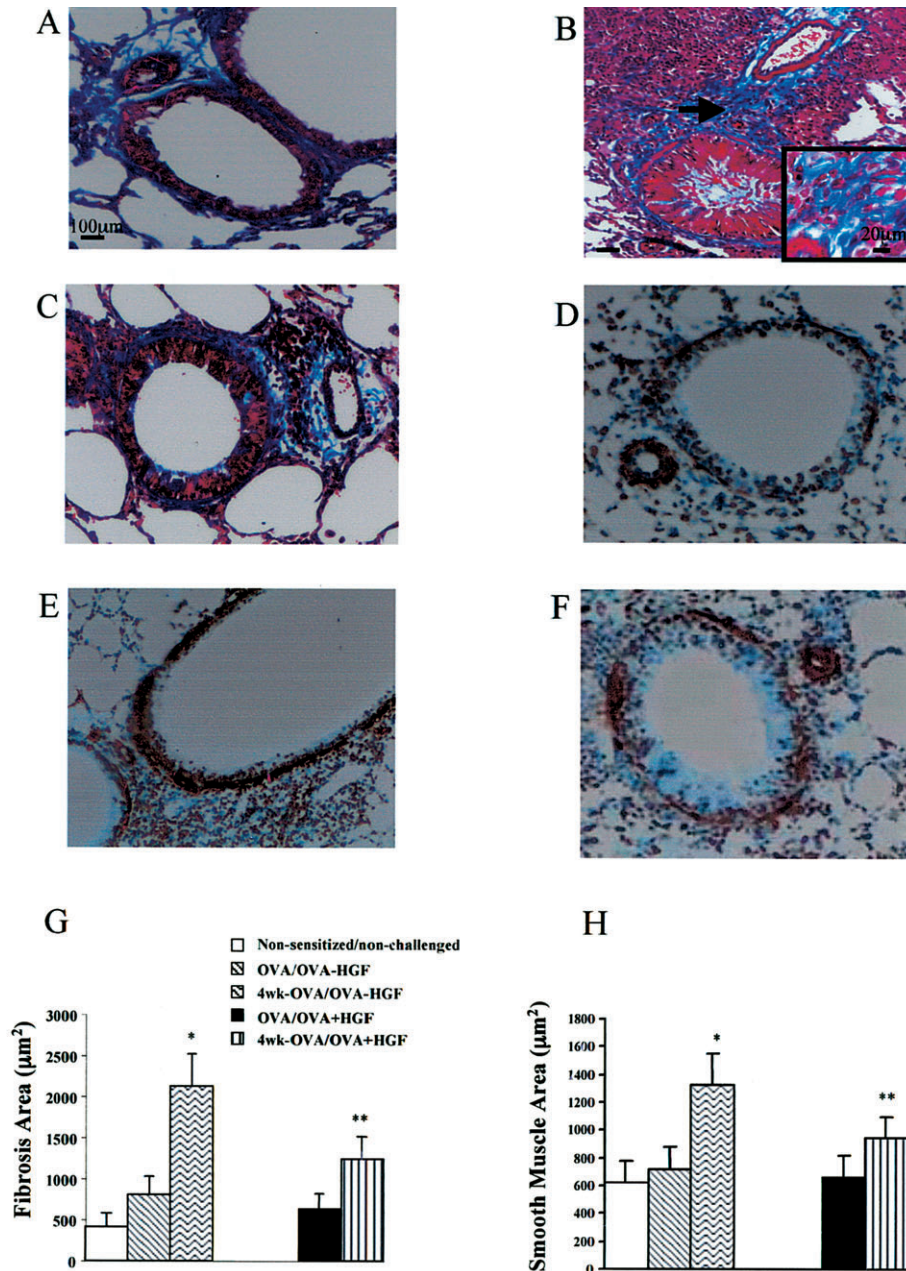


Figure 6. Treatment with HGF inhibits airway remodeling in mice receiving repeated (4 wk) airway challenges. The area of Masson's trichrome-positive peribronchiolar collagen layer (A–C, G) and α -SMA-stained smooth muscle layer (D–F, H) was measured as described in MATERIALS AND METHODS (final magnification: $\times 400$; inset: $\times 1,000$). (A, D) Nonsensitized and nonchallenged, (B, E) 4 wk-OVA/OVA-HGF, (C, F) 4 wk-OVA/OVA+HGF. Data represent the mean $\pm$ SD ($n = 16$ in each group). *Significant differences ($P < 0.05$) between nonsensitized/nonchallenged mice and OVA-sensitized and OVA chronic challenged mice (4 wk-OVA/OVA-HGF). **Significant differences ($P < 0.05$) between OVA-sensitized/OVA-4 wk challenged mice (receiving saline) (4 wk-OVA/OVA-HGF) and HGF-treated mice (4 wk-OVA/OVA+HGF).

Another growth factor, HGF, is recognized as a multifunctional and essential cytokine, transducing signals for normal morphogenesis and regeneration. HGF functions as an anti-organotoxic agent in several organs (15, 47, 48). The *in vivo* effects of HGF on affected organs have been studied using animal models of acute hepatic injury, liver cirrhosis and renal failure (17, 19, 20). Strikingly, mRNA for HGF is expressed at high levels in the lung compared with other organs (49). Further, HGF is synthesized by a variety of cell types, including fibroblasts, macrophages and other inflammatory cells, smooth muscle cells, and epithelial cells. Because of this distribution, we determined whether HGF plays a role in the development AHR and airway inflammation. In a lung fibrosis model, Yaekashiwa and coworkers demonstrated that recombinant human exogenous HGF prevented the progression of bleomycin-induced murine lung fibrosis when administered simultaneously or in a delayed fashion (18). Dohi and coworkers recently reported that HGF significantly attenu-

ated collagen accumulation induced by bleomycin, determined by quantitation of hydroxyproline content and by scoring the extent of fibrosis (50). These results suggested that HGF may have a therapeutic benefit in reducing pulmonary fibrosis.

Although inflammation is undoubtedly a cornerstone of asthma, it is now clear that the asthmatic response is more complex, and that inflammation, which is characteristic of the asthmatic airway, may in time lead to a remodeling response. As a result, it has been proposed that the structural alterations induced during remodeling may play an important role in eliciting the airway functional changes. Although airway remodeling is difficult to define, subepithelial fibrosis, goblet cell hyperplasia and mucus hypersecretion, and myofibroblast hypertrophy have been identified as components of this response (7).

In the present study, we showed that short-term or repeated airway challenge of sensitized BALB/c mice triggered increases in airway responsiveness to inhaled MCh. Furthermore, eosinophil

TABLE 1. HEPATOCYTE GROWTH FACTOR LEVELS IN BRONCHOALVEOLAR LAVAGE FLUID

Group	HGF Levels in BALF (ng/ml)
Nonsensitized/nonchallenged	0.50 ± 0.16
OVA/OVA-HGF	3.80 ± 1.82*
2 wk-OVA/OVA-HGF	6.77 ± 1.08†
4 wk-OVA/OVA-HGF	2.06 ± 0.76‡
4 wk-OVA/OVA+HGF	4.38 ± 0.44§
OVA/OVA + anti-HGF	0.61 ± 0.17¶

Definition of abbreviations: BALF, bronchoalveolar lavage fluid; HGF, hepatocyte growth factor; OVA, ovalbumin.

* Significant differences ($P < 0.05$) between nonsensitized/nonchallenged mice and OVA-sensitized/OVA-challenged mice (OVA/OVA-HGF).

† Significant differences ($P < 0.05$) between nonsensitized/nonchallenged mice and OVA-sensitized and OVA chronic challenged mice.

‡ Significant differences ($P < 0.05$) between 2 wk-OVA/OVA-HGF mice and 4 wk-OVA/OVA-HGF mice.

§ Significant differences ($P < 0.05$) between 4 wk-OVA/OVA-HGF mice and 4 wk-OVA/OVA+HGF mice.

¶ Significant differences ($P < 0.05$) between OVA/OVA+anti-HGF mice and OVA/OVA+anti-HGF mice.

and lymphocyte accumulation in BAL fluid and lung tissue, and concentrations of the Th2 cytokines (IL-4, IL-5, and IL-13) and growth factors (TGF- β , PDGF, and NGF) in BAL fluid also increased when compared with nonsensitized and nonchallenged mice. The kinetics and maximum response for each of these parameters or AHR varied to some extent with the number of challenges. In parallel, histologic studies revealed that the numbers of cells staining positive for mucus, collagen deposition/fibrosis, and the thickness of the smooth muscle layer sur-

TABLE 2. SERUM OVA-SPECIFIC ANTIBODY LEVELS

Group	OVA-Specific IgE Levels (EU/ml)
Nonsensitized/nonchallenged	301 ± 33.0
OVA/OVA + saline	972 ± 320.8*
OVA/OVA + HGF	465 ± 156.4†

Definition of abbreviations: HGF, hepatocyte growth factor; OVA, ovalbumin.

* Significant differences ($P < 0.05$) between nonsensitized/nonchallenged mice and OVA-sensitized/OVA-challenged mice (OVA/OVA-HGF).

† Significant differences ($P < 0.05$) between OVA-sensitized/OVA-challenged control mice (receiving saline) and HGF-treated mice (OVA/OVA+HGF).

rounding the airway increased in sensitized mice exposed to repeated OVA challenges. These studies demonstrate that not only Th2 cytokines increase, but that a number of growth factors increase in parallel and have the potential to contribute to the pathogenesis of altered airway function. We demonstrate for the first time that the growth factor HGF is increased in the BAL fluid of sensitized and challenged mice, and that administration of exogenous HGF significantly attenuates increases in AHR, reduces the numbers of eosinophils and lymphocytes, and decreases levels of IL-4, IL-5, and IL-13 in the BAL fluid. In keeping with the marked reduction in Th2 cytokine levels, serum levels of OVA-specific IgE were also reduced. In contrast, IL-12, which is associated with induction of IFN- γ production and has suppressive effects on eosinophilopoiesis in bone marrow (51), was significantly increased after administration of HGF. Administration of exogenous HGF also inhibited PDGF and NGF levels in BAL fluid and reduced expression of TGF- β in the lung tissue of sensitized and challenged mice as well as TGF- β levels in the

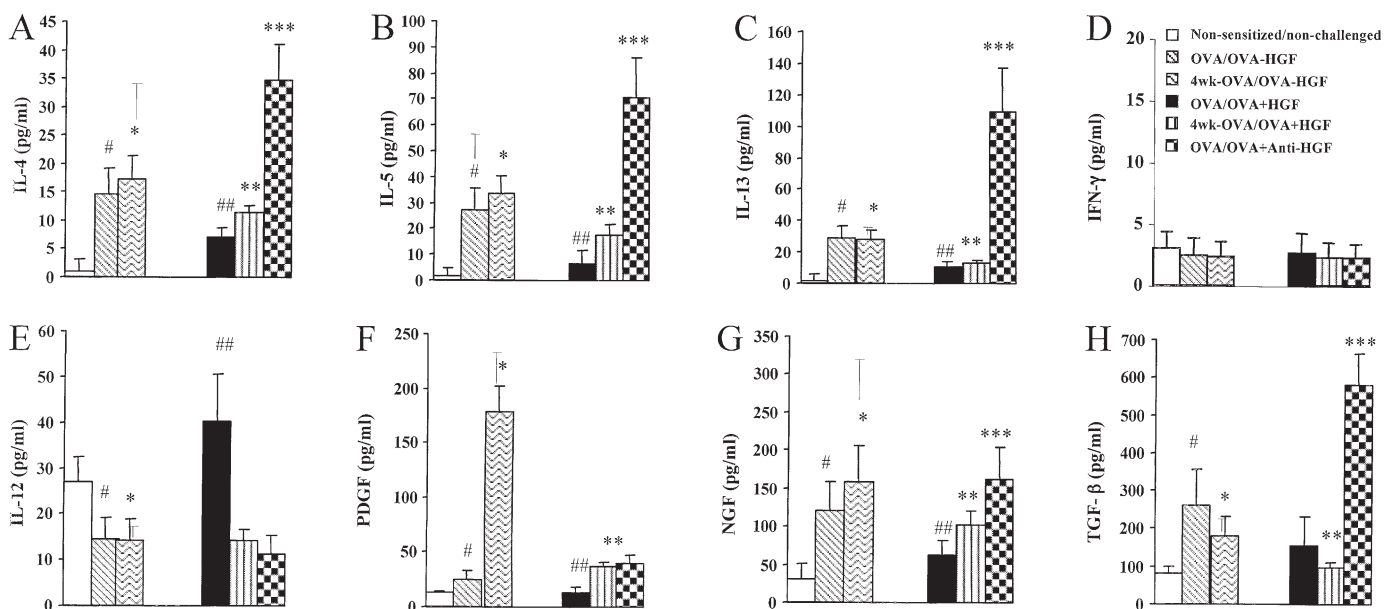


Figure 7. Treatment with HGF and anti-HGF alters cytokine and growth factor levels in BAL fluid. The levels (A) IL-4, (B) IL-5, (C) IL-13, (D) IFN- γ , (E) IL-12, (F) PDGF, (G) NGF, and (H) TGF- β in BAL fluid were measured as described in MATERIALS AND METHODS. The results for each group are expressed as the mean $\pm$ SD ($n = 16$ in each group). # Significant differences ($P < 0.05$) between nonsensitized/nonchallenged mice and OVA-sensitized/OVA-challenged mice (OVA/OVA-HGF). * Significant differences ($P < 0.05$) between nonsensitized/nonchallenged mice and OVA-sensitized and OVA chronic challenged mice (4 wk-OVA/OVA-HGF). ** Significant differences ($P < 0.05$) between OVA-sensitized/OVA-challenged mice (receiving saline) (OVA/OVA-HGF) and HGF-treated mice (OVA/OVA+HGF). *** Significant differences ($P < 0.05$) between OVA-sensitized/OVA-4 wk challenged mice (receiving saline) (4 wk-OVA/OVA-HGF) and HGF-treated mice (4 wk-OVA/OVA+HGF). **** Significant differences ($P < 0.05$) between OVA-sensitized/OVA-challenged mice (receiving IgG) and anti-HGF-treated mice (OVA/OVA+anti-HGF).

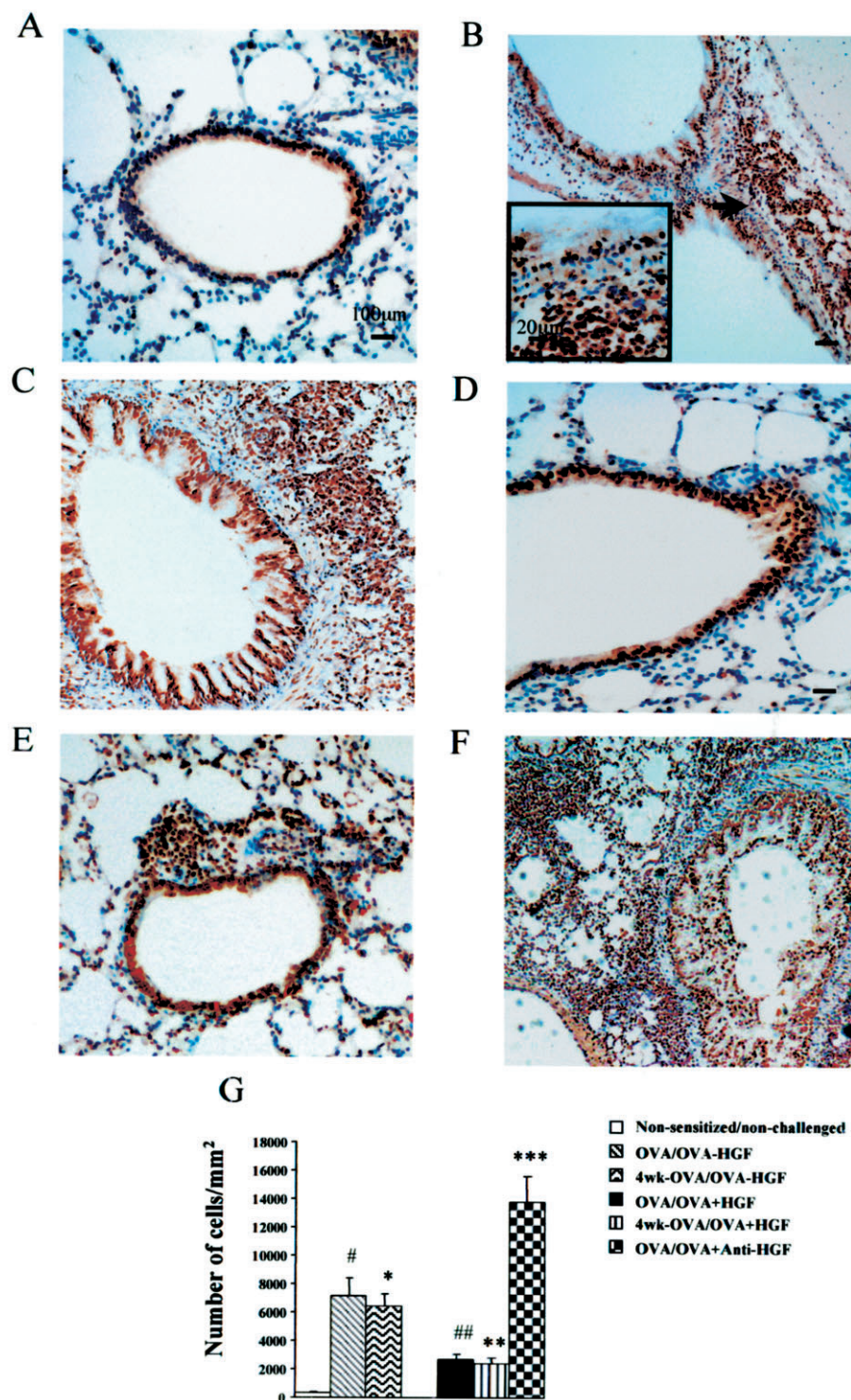


Figure 8. Staining of TGF- β in lung tissue. Immunohistochemical staining of TGF- β in the peribronchial and perivascular tissue after OVA sensitization and challenge was assessed as described in MATERIALS AND METHODS (final magnification: $\times 400$; inset: $\times 1,000$). (A) Nonsensitized and nonchallenged, (B) OVA/OVA-HGF, (C) 4 wk-OVA/OVA-HGF, (D) OVA/OVA+HGF, (E) 4 wk-OVA/OVA+HGF, (F) OVA/OVA+anti-HGF, (G) quantitative analysis of the number of TGF- β -positive cells in lung tissue.

BAL fluid. The effects of HGF on reducing IL-13 may account for the reduction in goblet cell hyperplasia and mucus hyperproduction (52, 53). It has been reported that IL-5 and IL-13 are involved in the development of subepithelial fibrosis in mice (54). The reduction of these cytokines following HGF administration may similarly account for the decreases in fibrosis observed.

To corroborate the studies on exogenous HGF administration, mice received intraperitoneal injections of neutralizing anti-HGF. Neutralization of endogenous HGF resulted in enhanced development of AHR and increased numbers of eosinophils in

BAL fluid. Furthermore, anti-HGF significantly increased the levels of Th2 cytokines (IL-4, IL-5, and IL-13) and growth factors (NGF and TGF- β) in BAL fluid. In the short-term model, anti-HGF also resulted in PAS+ cell numbers and collagen deposition. Thus, administration of exogenous HGF suppressed airway remodeling as well as airway inflammation and AHR, whereas neutralization of endogenous HGF enhanced airway inflammation and AHR. Neutralization of HGF was accompanied by marked increases in TGF- β . Cumulatively, these findings are in keeping with the notion that endogenous HGF can protect

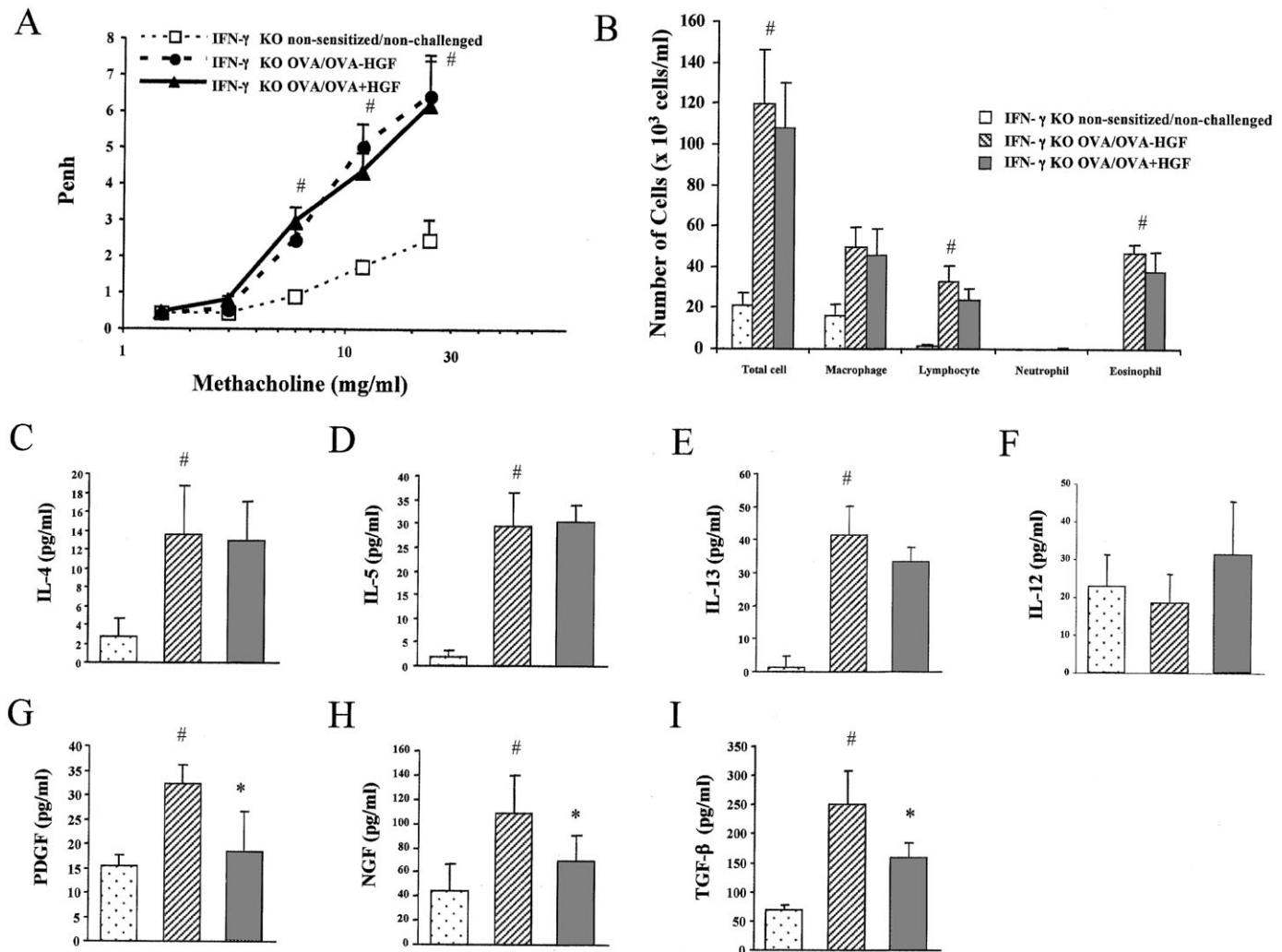


Figure 9. Effect of HGF in IFN- γ -deficient mice. AHR (A), the number of inflammatory cells (B), and IL-4 (C), IL-5 (D), IL-13 (E), IL-12 (F), PDGF (G), NGF (H), and TGF- β (I) levels in BAL fluid. The results for each group are expressed as the mean $\pm$ SD ($n = 16$ in each group). # Significant differences ($P < 0.05$) between nonsensitized/nonchallenged IFN- γ -deficient mice and OVA-sensitized/OVA-challenged IFN- γ -deficient mice (IFN- γ KO OVA/OVA-HGF). * Significant differences ($P < 0.05$) between OVA-sensitized/OVA-challenged IFN- γ -deficient mice (receiving saline) (IFN- γ KO OVA/OVA-HGF), and HGF-treated IFN- γ -deficient mice (IFN- γ KO OVA/OVA+HGF).

against the maintenance and progression of disease, whereas decreases in lung HGF levels may enhance the process contributing to airway remodeling.

In an attempt to define the mechanism whereby HGF exerts its effect on these different parameters, IFN- γ -deficient mice were studied. Recent studies showed that IFN- γ might prevent persistence of airway inflammation induced by acute allergen challenge (39, 40). The interaction between HGF and IFN- γ is somewhat controversial. Nagahori and coworkers (41) suggested that IFN- γ upregulates the HGF receptor in alveolar epithelial cells. In contrast, Takami and colleagues (55) reported that IFN- γ inhibits HGF-stimulated proliferation of bronchial epithelial cells. Differences in the levels of IFN- γ were not detected in the BAL fluid between any of the study groups, including the HGF-treated mice. Interestingly, administration of exogenous HGF to OVA-sensitized and -challenged IFN- γ -deficient mice failed to impact the elevated IL-4, IL-5, and IL-13 levels in BAL fluid or the development of AHR, in striking contrast to IFN- γ -sufficient mice. Exogenous HGF did reduce PDGF, NGF, and TGF- β levels in the BAL fluid of these IFN- γ -deficient mice, but

to a lesser degree than in IFN- γ -sufficient mice. The apparent dissociation of the effects of HGF in IFN- γ -deficient mice is unexplained at this time. These data suggest that HGF-mediated regulation of AHR, lung inflammation, and Th2 cytokine production are, at least in part, dependent on IFN- γ .

Recently, Liu and coworkers reported the rapid induction of renal HGF and HGF receptor (c-met) mRNA, beginning 1 h after acute renal damage, and that HGF protein was significantly decreased at 24 h in acute renal failure (56). TGF- β is a strong suppressor of HGF gene expression in human lung fibroblasts (57), and Mizuno and coworkers demonstrated that renal HGF levels were markedly decreased in the late stages of a murine model of chronic renal disease, whereas renal TGF- β levels were significantly increased in association with expansion of fibrotic areas (13, 27). In addition, treatment with exogenous HGF promoted renal epithelial cell proliferation and accelerated recovery from ischemic or toxic injury in rats (20, 58). Cumulatively, these data support the concept that the levels or ratio of HGF/TGF- β may play an important role in the balance of injury and repair.

Changes in the airways consistent with airway remodeling

were observed as the number of challenges increased. After sensitization and short-term or repeated challenges with OVA, BAL HGF levels increased; this was associated with increases in TGF- β levels, which persisted while HGF levels began to decrease. Treatment with exogenous HGF in mice increased BAL HGF levels and inhibited TGF- β levels in BAL fluid. Further, neutralization of endogenous HGF in sensitized and challenged mice was accompanied by increased TGF- β levels in BAL fluid. This relationship between HGF and TGF- β may be important in the balance between tissue repair and airway remodeling in the lung in asthma, especially after repeated allergen exposure and exacerbations. In this murine model, we demonstrate the important role of endogenous HGF in protecting the airways, and reducing inflammation, AHR, and airway remodeling. The absence of HGF may lead to the overexpression of TGF- β , whereas supplementing HGF (exogenously) may attenuate the onset of airway inflammation and airway remodeling, as has been observed with liver cirrhosis (12, 17), renal fibrosis (13), and pulmonary fibrosis (18).

In summary, HGF is prominently expressed in lung tissue and endogenous levels increase in response to challenge of sensitized mice. Repeated allergen challenge is associated with an increase in TGF- β . After administration of exogenous HGF, many of the responses viewed as important in the development of allergic airway inflammation, AHR, and airway remodeling are significantly reduced. For these reasons, HGF appears to be an important regulator of allergic airway inflammation and AHR and may have potential in the treatment of chronic allergic asthma.

Acknowledgments: The authors thank D. Nabighian for her assistance in preparation of this manuscript.

References

- Azzawi M, Bradley B, Jeffery PK, Frew AJ, Wardlaw AJ, Knowles G, Assoufi B, Collins JV, Durham S, Kay AB. Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. *Am Rev Respir Dis* 1990;142:1407-1413.
- Ying S, Humbert M, Barkans J, Corrigan CJ, Pfister R, Menz G, Larche M, Robinson DS, Durham SR, Kay AB. Expression of IL-4 and IL-5 mRNA and protein product by CD4+ and CD8+ T cells, eosinophils, and mast cells in bronchial biopsies obtained from atopic and nonatopic (intrinsic) asthmatics. *J Immunol* 1997;158:3539-3544.
- Kotsimbos TC, Ernst P, Hamid QA. Interleukin-13 and interleukin-4 are coexpressed in atopic asthma. *Proc Assoc Am Physicians* 1996;108:368-378.
- Minty A, Asselin S, Bensussan A, Shire D, Vita N, Vyakarnam A, Wijdenes J, Ferrara P, Caput D. The related cytokines interleukin-13 and interleukin-4 are distinguished by differential production and differential effects on T lymphocytes. *Eur Cytokine Netw* 1997;8:203-213.
- Hogan SP, Matthei KI, Young JM, Koskinen A, Young IG, Foster PS. A novel T cell-regulated mechanism modulating allergen-induced airways hyperactivity in BALB/c mice independently of IL-4 and IL-5. *J Immunol* 1998;161:1501-1509.
- Hansen G, Berry G, DeKruyff RH, Umetsu DT. Allergen-specific Th1 cells fail to counterbalance Th2 cell-induced airway hyperactivity but cause severe airway inflammation. *J Clin Invest* 1999;103:175-183.
- Elias JA, Zhu Z, Chupp G, Homer RJ. Airway remodeling in asthma. *J Clin Invest* 1999;104:1001-1006.
- Vignola AM, Chanez P, Chiappara G, Merendino A, Pace E, Rizzo A, Rocca AM, Bellia V, Bonsignore G, Bousquet J. Transforming growth factor- β expression in mucosal biopsies in asthma and chronic bronchitis. *Am J Respir Crit Care Med* 1997;156:591-599.
- Buhling F, Tholert G, Kaiser D, Hoffmann B, Reinhold D, Ansoorge S, Welte T. Increased release of transforming growth factor (TGF)- β 1, TGF- β 2, and chemoattractant mediators in pneumonia. *J Interferon Cytokine Res* 1999;19:271-278.
- Nakamura T, Nawa K, Ichihara A, Kaise N, Nishino T. Purification and subunit structure of hepatocyte growth factor from rat platelets. *FEBS Lett* 1987;224:311-316.
- Matsumoto K, Nakamura T. Emerging multipotent aspects of hepatocyte growth factor. *J Biochem* 1996;119:591-600.
- Ueki T, Kaneda Y, Tsutsui H, Nakanishi K, Sawa Y, Morishita R, Matsumoto K, Nakamura T, Takahashi H, Okamoto E, *et al.* Hepatocyte growth factor gene therapy of liver cirrhosis in rats. *Nat Med* 1999;5:226-230.
- Mizuno S, Kurosawa T, Matsumoto K, Mizuno-Horikawa Y, Okamoto M, Nakamura T. Hepatocyte growth factor prevents renal fibrosis and dysfunction in a mice model of chronic renal disease. *J Clin Invest* 1998;101:1827-1834.
- Taniyama Y, Morishita R, Nakagami H, Moriguchi A, Sakonjo H, Shokei-Kim, Matsumoto K, Nakamura T, Higaki J, Ogihara T. Potential contribution of a novel antifibrotic factor, hepatocyte growth factor, to prevention of myocardial fibrosis by angiotensin II blockade in cardiomyopathic hamsters. *Circulation* 2000;102:246-252.
- Schmidt C, Bladt F, Goedecke S, Brinkmann V, Zschiesche W, Sharpe M, Gherardi ME, Birchmeier C. Scatter factor/hepatocyte growth factor is essential for liver development. *Nature* 1995;373:699-702.
- Uehara Y, Minowa O, Mori C, Shiota K, Kuno J, Noda T, Kitamura N. Placental defect and embryonic lethality in mice lacking hepatocyte growth factor/scatter factor. *Nature* 1995;373:702-705.
- Matsuda Y, Matsumoto K, Ichida T, Nakamura T. Hepatocyte growth factor suppresses the onset of liver cirrhosis and abrogates lethal hepatic dysfunction in rats. *J Biochem* 1995;118:643-649.
- Yaekashiwa M, Nakayama S, Ohnuma K, Sakai T, Abe T, Satoh K, Matsumoto K, Nakamura T, Takahashi T, Nukiwa T. Simultaneous or delayed administration of hepatocyte growth factor equally represses the fibrotic changes in murine lung injury induced by bleomycin. *Am J Respir Crit Care Med* 1997;156:1937-1944.
- Ishiki Y, Ohnishi H, Muto Y, Matsumoto K, Nakamura T. Direct evidence that hepatocyte growth factor is a hepatotrophic factor for liver regeneration and has a potent antihepatitis effect in vivo. *Hepatology* 1992;16:1227-1235.
- Kawaida K, Matsumoto K, Shimazu H, Nakamura T. Hepatocyte growth factor prevents acute renal failure and accelerates renal regeneration in mice. *Proc Natl Acad Sci USA* 1994;91:4357-4361.
- Yanagita K, Matsumoto K, Sekiguchi K, Ishibashi H, Niho Y, Nakamura T. Hepatocyte growth factor may act as a pulmotrophic factor on lung regeneration after acute lung injury. *J Biol Chem* 1993;268:21212-21217.
- Lahn M, Kanehiro A, Takeda K, Joetham A, Schwarze J, Kohler G, O'Brien R, Gelfand EW, Born W. Negative regulation of airway hyperresponsiveness that is dependent on $\gamma\delta$ T cells and independent of $\alpha\beta$ T cells. *Nat Med* 1999;5:1150-1156.
- Kanehiro A, Takeda K, Joetham A, Tomkinson A, Ikemura T, Irvin CG, Gelfand EW. Timing of administration of anti-VLA-4 differentiates airway hyperresponsiveness in the central and peripheral airways in mice. *Am J Respir Crit Care Med* 2000;162:1132-1139.
- Seki T, Ihara I, Sugimura A, Shimonishi M, Nishizawa T, Asami O, Hagiya M, Nakamura T, Shimizu S. Isolation and expression of cDNA for different forms of hepatocyte growth factor from human leukocytes. *Biochem Biophys Res Commun* 1990;172:321-327.
- Santos OF, Barros EJ, Yang XM, Matsumoto K, Nakamura T, Park M, Nigam SK. Involvement of hepatocyte growth factor in kidney development. *Dev Biol* 1994;163:525-529.
- Ohmichi H, Koshimizu U, Matsumoto K, Nakamura T. Hepatocyte growth factor (HGF) acts as a mesenchyme-derived morphogenic factor during fetal lung development. *Development* 1998;125:1315-1324.
- Mizuno S, Matsumoto K, Kurosawa T, Mizuno-Horikawa Y, Nakamura T. Reciprocal balance of hepatocyte growth factor and transforming growth factor-beta 1 in renal fibrosis in mice. *Kidney Int* 2000;57:937-948.
- Sakamaki Y, Matsumoto K, Mizuno S, Miyoshi S, Matsuda H, Nakamura T. Hepatocyte growth factor stimulates proliferation of respiratory epithelial cells during postpneumectomy compensatory lung growth in mice. *Am J Respir Cell Mol Biol* 2002;26:525-533.
- Hamelmann E, Schwarze J, Takeda K, Oshiba A, Larsen GL, Irvin CG, Gelfand EW. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am J Respir Crit Care Med* 1997;156:766-775.
- Takeda K, Hamelmann E, Joetham A, Shultz LD, Larsen GL, Irvin CG, Gelfand EW. Development of eosinophilic airway inflammation and airway hyperresponsiveness in mast cell-deficient mice. *J Exp Med* 1997;186:449-454.
- Temann UA, Prasad B, Gallup MW, Basbaum C, Ho SB, Flavell RA, Rankin JA. A novel role for murine IL-4 in vivo: induction of MUC5AC gene expression and mucin hypersecretion. *Am J Respir Cell Mol Biol* 1997;16:471-478.
- Henderson WR Jr, Tang LO, Chu SJ, Tsao SM, Chiang GK, Jones F, Jonas M, Pae C, Wang H, Chi EY. A role for cysteinyl leukotrienes

- in airway remodeling in a mouse asthma model. *Am J Respir Crit Care Med* 2002;165:108–116.
33. Bonner JC, Rice AB, Moomaw CR, Morgan DL. Airway fibrosis in rats induced by vanadium pentoxide. *Am J Physiol Lung Cell Mol Physiol* 2000;278:L209–L216.
34. Kuhn C III, Homer RJ, Zhu Z, Ward N, Flavell RA, Geba GP, Elias JA. Airway hyperresponsiveness and airway obstruction in transgenic mice: morphologic correlates in mice overexpressing interleukin (IL)-11 and IL-6 in the lung. *Am J Respir Cell Mol Biol* 2000;22:289–295.
35. Yo Y, Morishita R, Yamamoto K, Tomita N, Kida I, Hayashi S, Moriguchi A, Kato S, Matsumoto K, Nakamura T, et al. Actions of hepatocyte growth factor as a local modulator in the kidney: potential role in pathogenesis of renal disease. *Kidney Int* 1998;53:50–58.
36. Morishita R, Nakamura S, Nakamura Y, Aoki M, Moriguchi A, Kida I, Yo Y, Matsumoto K, Nakamura T, Higaki J, et al. Potential role of an endothelium-specific growth factor, hepatocyte growth factor, on endothelial damage in diabetes. *Diabetes* 1997;46:138–142.
37. Eddy AA. Molecular insights into renal interstitial fibrosis. *J Am Soc Nephrol* 1996;7:2495–2508.
38. Border WA, Noble NA. Transforming growth factor beta in tissue fibrosis. *N Engl J Med* 1994;331:1286–1292.
39. Yoshida M, Leigh R, Matsumoto K, Wattie J, Ellis R, O'Byrne PM, Inman MD. Effect of interferon- γ on allergic airway responses in interferon- γ -deficient mice. *Am J Respir Crit Care Med* 2002;166:451–456.
40. Hofstra CL, Van Ark I, Hofman G, Nijkamp FP, Jardieu PM, Van Oosterhout AJ. Differential effects of endogenous and exogenous interferon- γ on immunoglobulin E, cellular infiltration, and airway responsiveness in a murine model of allergic asthma. *Am J Respir Cell Mol Biol* 1998;19:826–835.
41. Nagahori T, Dori M, Matsumoto K, Saitoh K, Honda ZI, Nakamura T, Yamamoto K. Interferon-gamma upregulates the c-Met/hepatocyte growth factor expression in alveolar epithelial cells. *Am J Respir Cell Mol Biol* 1999;21:490–497.
42. Minshall EM, Leung DYM, Martin RJ, Song YL, Cameron L, Ernst P, Hamid Q. Eosinophil-associated TGF- β 1 mRNA expression and airways fibrosis in bronchial asthma. *Am J Respir Cell Mol Biol* 1997;17:326–333.
43. Yamashita N, Sekine K, Miyasaka T, Kawashima R, Nakajima Y, Nakano J, Yamamoto T, Horiuchi T, Hirai K, Ohta K. Platelet-derived growth factor is involved in the augmentation of airway responsiveness through remodeling of airways in the diesel exhaust particulate-treated mice. *J Allergy Clin Immunol* 2001;107:135–142.
44. Fox AJ, Patel HJ, Barnes J, Belvisi MG. Release of nerve growth factor by human pulmonary epithelial cells: role in airway inflammatory diseases. *Eur J Pharmacol* 2001;424:159–162.
45. Olgart C, Frossard N. Human lung fibroblasts secrete nerve growth factor: effect of inflammatory cytokines and glucocorticoids. *Eur Respir J* 2001;18:115–121.
46. Braun A, Quarcoo D, Schulte-Herbruggen O, Lommatzsch M, Hoyle G, Renz H. Nerve growth factor induces airway hyperresponsiveness in mice. *Int Arch Allergy Immunol* 2001;122:205–207.
47. Boros P, Miller CM. Hepatocyte growth factor: a multifunctional cytokine. *Lancet* 1995;345:293–295.
48. Zarnegar R, Michalopoulos GK. The many faces of hepatocyte growth factor: from hepatopoiesis to hematopoiesis. *J Cell Biol* 1995;129:1177–1180.
49. Tashiro K, Hagiya M, Nishizawa T, Seki T, Shimonishi M, Shimizu S, Nakamura T. Deduced primary structure of rat hepatocyte growth factor and expression of the mRNA in rat tissues. *Proc Natl Acad Sci USA* 1990;87:3200–3204.
50. Dohi M, Hasegawa T, Yamamoto K, Marshall BC. Hepatocyte growth factor attenuates collagen accumulation in a murine model of pulmonary fibrosis. *Am J Respir Crit Care Med* 2000;162:2302–2307.
51. Rais M, Wild JS, Choudhury BK, Alam R, Stafford S, Dharajiya N, Sur S. Interleukin-12 inhibits eosinophil differentiation from bone marrow stem cells in an interferon-gamma-dependent manner in a mouse model of asthma. *Clin Exp Allergy* 2002;32:627–632.
52. Tomkinson A, Kanehiro A, Rabinovitch N, Joetham A, Cieslewicz G, Gelfand EW. The failure of STAT6-deficient mice to develop airway eosinophilia and airway hyperresponsiveness is overcome by interleukin-5. *Am J Respir Crit Care Med* 1999;160:1281–1291.
53. Mattes J, Yang M, Siqueira A, Clark K, MacKenzie J, McKenzie AN, Webb DC, Matthei KI, Foster PS. IL-13 induces airway hyperactivity independently of the IL-4R α chain in the allergic lung. *J Immunol* 2001;167:1683–1692.
54. Lee JJ, McGarry MP, Farmer SC, Denzler KL, Larson KA, Carrigan PE, Brenneise IE, Horton MA, Haczu A, Gelfand EW, et al. Interleukin-5 expression in the lung epithelium of transgenic mice leads to pulmonary changes pathognomonic of asthma. *J Exp Med* 1997;185:2143–2156.
55. Takami K, Takuwa N, Okazaki H, Kobayashi M, Ohtoshi T, Kawasaki S, Dohi M, Yamamoto K, Nakamura T, Tanaka M, et al. Interferon-gamma inhibits hepatocyte growth factor-stimulated cell proliferation of human bronchial epithelial cells: upregulation of p27(kip1) cyclin-dependent kinase inhibitor. *Am J Respir Cell Mol Biol* 2002;26:231–238.
56. Liu Y, Tolbert EM, Lin L, Thursby MA, Sun AM, Nakamura T, Dworkin LD. Up-regulation of hepatocyte growth factor receptor: an amplification and targeting mechanism for hepatocyte growth factor action in acute renal failure. *Kidney Int* 1999;55:442–453.
57. Matsumoto K, Tajima H, Okazaki H, Nakamura T. Negative regulation of hepatocyte growth factor gene expression in human lung fibroblasts and leukemic cells by transforming growth factor- β 1 and glucocorticoids. *J Biol Chem* 1992;267:24917–24920.
58. Miller SB, Martin DR, Kissane J, Hammerman MR. Hepatocyte growth factor accelerates recovery from acute ischemic renal injury in rats. *Am J Physiol* 1994;266:F129–F134.