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A Mouse Model of Airway Disease: Oncostatin M-Induced Pulmonary Eosinophilia, Goblet Cell Hyperplasia, and Airway Hyperresponsiveness Are STAT6 Dependent, and Interstitial Pulmonary Fibrosis Is STAT6 Independent

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Oncostatin M (OSM), a pleiotropic cytokine of the gp130 cytokine family, has been implicated in chronic allergic inflammatory and fibrotic disease states associated with tissue eosinophilia. Mouse (m)OSM induces airway eosinophilic inflammation and interstitial pulmonary fibrosis in vivo and regulates STAT6 activation in vitro. To determine the requirement of STAT6 in OSM-induced effects in vivo, we examined wild-type (WT) and STAT6-knockout (STAT6^{-/-}) C57BL/6 mouse lung responses to transient ectopic overexpression of mOSM using an adenoviral vector (AdmOSM). Intratracheal AdmOSM elicited persistent eosinophilic lung inflammation that was abolished in STAT6^{-/-} mice. AdmOSM also induced pronounced pulmonary remodeling characterized by goblet cell hyperplasia and parenchymal interstitial fibrosis. Goblet cell hyperplasia was STAT6 dependent; however, parenchymal interstitial fibrosis was not. OSM also induced airway hyperresponsiveness in WT mice that was abolished in STAT6^{-/-} mice. OSM stimulated an inflammatory signature in the lungs of WT mice that demonstrated STAT6-dependent regulation of Th2 cytokines (IL-4, IL-13), chemokines (eotaxin-1/2, MCP-1, keratinocyte chemoattractant), and extracellular matrix modulators (tissue inhibitor of matrix metalloproteinase-1, matrix metalloproteinase-13), but STAT6-independent regulation of IL-4Rα, total lung collagen, collagen-1A1, -1A2 mRNA, and parenchymal collagen and α smooth muscle actin accumulation. Thus, overexpression of mOSM induces STAT6-dependent pulmonary eosinophilia, mucous/goblet cell hyperplasia, and airway hyperresponsiveness but STAT6-independent mechanisms of lung tissue extracellular matrix accumulation. These results also suggest that eosinophil or neutrophil accumulation in mouse lungs is not required for OSM-induced lung parenchymal collagen deposition and that OSM may have unique roles in the pathogenesis of allergic and fibrotic lung disease. *The Journal of Immunology*, 2011, 186: 000–000.

Oncostatin M (OSM), a member of the gp130 cytokine family (1–3), has recently been shown to be upregulated in tissues of various allergic and fibrotic disease states associated with eosinophilic inflammation including allergic dermatitis (4), perennial allergic rhinitis (5), and idiopathic pulmonary fibrosis (IPF) (6). It has also been proposed that OSM may be

important in the pathogenesis of asthma, particularly in the airway remodeling response (7). A common theme in connective tissue remodeling and homeostasis is the balance between the actions of matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs). Oncostatin M has a prominent role in the regulation of connective tissue extracellular matrix (ECM) through regulation of MMPs and TIMPs (8–12). Utilizing an adenoviral vector (Ad) overexpression system, we previously demonstrated that transient ectopic expression of mouse (m)OSM (AdmOSM) in the lungs of mice leads to ECM accumulation (13, 14). AdmOSM also elicits a robust eosinophilic airway inflammation (14), which is consistent with OSM's ability to regulate eotaxin-1 expression in airway fibroblasts (14, 15) and airway smooth muscle cells (16) in vitro. A recent study (6) has shown that administration of rmOSM to mouse lungs also induces similar effects as those of AdmOSM. Among the gp130 cytokine family members, our studies implicate OSM as a unique inducer of signaling/transcription factor STAT6 activation in mouse lung fibroblasts (15). STAT6 appears to regulate collagen synthesis (17) and has been implicated in fibrosis in an experimental model of scleroderma (18). In addition, OSM cooperates with IL-4 (15, 16) and IL-13 (16) in promoting enhanced eotaxin-1 responses in a STAT6-dependent fashion (15). However, whether STAT6-mediated responses in vitro reflect responses to OSM in vivo is not known.

STAT6 has been primarily associated with cellular responses to IL-4 and IL-13 cytokines, which are critical for Th2 immune responses. Several clinical studies have shown elevated expression of IL-4 and IL-13 in subjects with allergic asthma (19) and IPF (20,

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Abbreviations used in this article: Ad, adenoviral vector; AdDI70, empty control vector; AdmOSM, adenoviral vector of mouse oncostatin M; AHR, airway hyperresponsiveness; BAL, bronchoalveolar lavage; ECM, extracellular matrix; G, tissue resistance/damping; H, tissue elastance/stiffness; IPF, idiopathic pulmonary fibrosis; KC, keratinocyte chemoattractant; KO, knockout; MCh, methacholine; mdc, minimum detection concentration; MMP, matrix metalloproteinase; MNC, mononuclear cell; mOSM, mouse oncostatin M; OSM, oncostatin M; PAS, periodic acid-Schiff; PSR, Picosirius red; Rn, airway resistance; αSMA, α smooth muscle actin; STAT6^{-/-}, STAT6-knockout; TCN, total cell number; TIMP, tissue inhibitor of matrix metalloproteinase; WT, wild-type.

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21). Experimental models of allergic airway disease have demonstrated that both IL-4 and IL-13 play an essential role in the development of various attributes of the human asthma phenotype including subepithelial fibrosis, mucous/goblet cell hyperplasia, and airway hyperresponsiveness (AHR) (22–26). In an animal model of bleomycin-induced pulmonary fibrosis, both IL-4 and IL-13 are expressed at local inflammatory tissue sites (27–29) and appear to participate in maintaining the pulmonary interstitial fibrotic phenotype (27). IL-4 and IL-13 mediate their biological responses through receptor complexes that share the IL-4R α -chain (30) from which the STAT6 pathway is activated (31). The IL-4R α –STAT6 axis has been shown to be critical in the development of the asthma phenotype in animal models of acute allergic airway disease, as neutralization of STAT6 (32–34) or IL-4R α (22, 35) inhibits airway eosinophilia, airway remodeling, and AHR. However, STAT6-independent inflammatory processes can also be induced in models of allergic airway disease (36).

Accumulation of inflammatory cells including eosinophils is thought to contribute to subepithelial basement membrane remodeling (37, 38) and AHR (39, 40) in asthmatics and pulmonary fibrosis and reduced diffusion capacity (41, 42) in subjects with IPF. Eosinophil migration in animal models of asthma (43–45) and IPF (46, 47) is mediated by the cooperation of the eosinophil-selective chemokines (eotaxin-1/2) and Th2 cytokines (IL-5, IL-4, IL-13). IL-5 is important in eosinophil differentiation (48) and mobilization into the circulation from the bone marrow, whereas eotaxins establish a chemotactic gradient at local tissue sites for eosinophil recruitment (49). Because eotaxins are regulated by IL-4 and IL-13 in a STAT6-dependent fashion in various airway structural cells (15, 16, 50, 51), this provides a mechanism by which Th2 cytokines in concert with eotaxin chemokines promote local tissue eosinophilia during inflammatory disease states.

In this study, we aimed to investigate the role of the STAT6 pathway in AdmOSM-induced pulmonary inflammation and remodeling. We observed that the wild-type (WT) mouse lungs treated with AdmOSM developed pronounced airway and tissue eosinophilia, mucous/goblet cell hyperplasia, interstitial pulmonary fibrosis, and AHR in response to methacholine (MCh). AdmOSM was able to induce an inflammatory signature marked by Th2 cytokine (IL-4, IL-13, IL-5), chemokine (eotaxin-1/2, MCP-1, keratinocyte chemoattractant [KC]), IL-4R α , TIMP-1, MMP-13, and tissue ECM (collagen, α smooth muscle actin [α SMA]) expression. Administration of AdmOSM into STAT6-knockout (STAT6^{-/-}) mouse lungs revealed that STAT6 is required for mOSM-induced pulmonary eosinophilia, mucous/goblet cell hyperplasia, AHR, and chemokine and Th2 cytokine expression. In this system, STAT6 and eosinophil infiltration into mouse lungs appears dispensable for lung parenchymal remodeling.

Materials and Methods

Animals

Female C57BL/6 mice (8–10 wk old) were purchased from Charles River Laboratories (Ottawa, Ontario, Canada). Female STAT6 homozygous knockout (STAT6^{-/-}) mice on a C57BL/6 background were bred in-house, courtesy of Dr. Waliul I. Khan (McMaster University, Hamilton, Ontario, Canada). All mice were housed under specific pathogen-free conditions and maintained on a 12-h light/dark cycle, with food and water ad libitum. Mice were acclimatized for at least 1 wk and experiments performed on 10–12-wk-old mice. All experimental procedures described in this manuscript were approved by the Animal Research Ethics Board of McMaster University.

Administration of adenoviral constructs

WT (STAT6^{+/+}) and STAT6^{-/-} mice were administered with 5×10^7 PFU replication-deficient Ad that expresses mouse OSM (AdmOSM) or an

empty control vector (Add170) through the endotracheal route (by intubation) in a 60 μ l volume of sterile PBS. All animal procedures were performed with inhalation anesthesia with isoflurane.

Sample collection and measurement

Mice were euthanized by dissecting the abdominal aorta 7 or 14 d post endotracheal Ad administration, and bronchoalveolar lavage (BAL) was performed. Briefly, the trachea was cannulated with a polyethylene tube (BD Biosciences) and secured by a string. Lungs were dissected en bloc and lavaged two times with a total volume of 600 μ l PBS (400 μ l followed by 200 μ l). Approximately 350 μ l instilled fluid was consistently removed and subjected to centrifugation at 1500 rpm for 5 min followed by a pulse at 10,000 rpm for 6 s. After centrifugation, BAL supernatants were stored at -20°C for cytokine and chemokine analysis by LUMINEX or ELISA (see below); cell pellets were resuspended with 100 μ l PBS, total cell counts determined using a hemocytometer, and BAL smears prepared by cytocentrifugation at 300 rpm for 2 min. BAL smears were stained with Protocol Hema 3 stain set (Fisher Scientific) and differential cell counts (≥ 400 leukocytes) determined using standard hemocytologic criteria to classify leukocytes as neutrophils, eosinophils, or mononuclear cells (MNC). Following BAL, the right lung was dissected and either: 1) stored in RNAlater (Ambion) at -20°C for RNA analysis (TaqMan) (see below); or 2) placed into HBSS at 4°C for lung cell isolation and analysis by flow cytometry (see below); the left lung was perfused with 10% formalin at a constant pressure of 20 cm H₂O, fixed in 10% formalin for 48 h, and subsequently used for histology/immunohistochemistry (see below). For hematological analysis, peripheral blood was obtained, blood smears prepared and stained with Protocol Hema 3 stain set (Fisher Scientific), and differential cell counts (≥ 200 leukocytes) determined using standard hemocytologic criteria to classify leukocytes as neutrophils, eosinophils, or MNC.

Lung cell isolation

Once right lungs were dissected, they were cut into small pieces (~ 1 mm in diameter using scissors) and agitated at 37°C for 1 h in 4 ml 150 U/ml collagenase type III (Life Technologies, Burlington, Ontario, Canada) in HBSS. Lung pieces were then triturated through a 40- μ m cell strainer (BD Biosciences) using the plunger end of a 3 ml syringe into FACS buffer (0.5% BSA in PBS). Cells were then centrifuged at 1200 rpm for 10 min at 4°C and RBCs subsequently lysed using 1 ml ACK lysis buffer (0.5 M NH₄Cl, 10 mM KHCO₃, and 0.1 mM Na₂EDTA [pH 7.2–7.4]) for 1 min at room temperature. Lysis was neutralized with FACS buffer (0.5% BSA in PBS). Cells were washed once with FACS buffer, resuspended, and filtered again prior to staining for flow cytometric analysis. Cytospins were also prepared from an aliquot of the lung cell suspension for differential cell counting as described above for the BAL.

Flow cytometric analysis

To minimize nonspecific binding, cells were first incubated with FcBlock (anti-CD16/CD32; BD Pharmingen, Mississauga, Ontario, Canada) in FACS buffer for 15 min at 4°C . For each Ab combination, 2×10^6 cells were incubated with mAbs for 30 min at 4°C . Cells were then washed twice with FACS buffer. Data were collected using an LSRII (BD Biosciences) flow cytometer and analyzed using FlowJo software (Tree Star, Ashland, OR). The following Abs were used: anti-CD45 (allophycocyanin-Cy7-conjugated 30-F11 clone) purchased from BD Pharmingen; anti-GR-1 (PE-Cy5.5-conjugated RB6 8C5 clone) and anti-IgG_{2b} (PE-Cy5.5-conjugated) purchased from eBioscience (San Diego, CA). Isotype control was used for Gr-1, and a fluorescence minus one control was used for CD45. Abs were titrated to determine optimal concentration. Gating strategies employed in the analysis of flow cytometric data are shown in Supplemental Fig. 1.

Histology and immunohistochemistry

Following formalin fixation, the left lung was longitudinally trisected into superior, central, and inferior tissue segments, paraffin embedded, and each segment cut into 3- μ m-thick cross sections. Lung sections were stained with H&E to assess the severity of lung pathology and leukocyte infiltration, Picrosirius red (PSR) to assess collagen deposition, and periodic acid-Schiff (PAS) to assess mucous/goblet cells. Immunohistochemistry for α SMA was also performed.

Morphometric analysis

Photomicrographs of the main bronchus (central tissue segment) and parenchyma (superior tissue segment) were captured with OpenLab software (version 3.0.3; Improvision, Guelph, Ontario, Canada) using a Leica camera

and microscope (Leica Microsystems, Richmond Hill, Ontario, Canada). Morphometric analysis of photomicrographs was performed using a custom computerized analysis system (Northern Eclipse software version 5; Empix Imaging, Mississauga, Ontario, Canada) and performed as previously described (52). Briefly, morphometric analysis was performed by determining the percentage of positively stained tissue area within a software designated bandwidth area. The bandwidth area used to analyze each tissue remodeling response is as follows: 1) 36–38- μ m-thick bandwidth extending outward from the subepithelial basement membrane into the parenchyma of PSR-stained lung tissue cross sections was used to quantify subepithelial collagen deposition (main bronchus, middle portion of left lung, 20 $\times$ objective); 2) 26–28- μ m-thick bandwidth extending outward from the subepithelial basement membrane into the airway lumen of PAS-stained lung tissue cross sections was used to quantify mucous/goblet cells (main bronchus, middle portion of left lung, 20 $\times$ objective); 3) 900- μ m-thick bandwidth extending outward from the periphery of the tunica adventitia that surrounds the main bronchial airway into the parenchyma of PSR-stained lung tissue cross-sections was used to quantify parenchymal collagen deposition (parenchyma, superior portion of left lung, 5 $\times$ objective); and 4) 20- μ m-thick bandwidth extending outward from the subepithelial basement membrane into the parenchyma of α SMA-immunostained lung tissue cross sections was used to quantify airway smooth muscle thickness (main bronchus, middle portion of left lung, 20 $\times$ objective).

RNA purification and analysis by RT-PCR (TaqMan)

The right lung was homogenized in TRIzol (Invitrogen Life Technologies) and total lung RNA extracted according to the manufacturer's instructions. RNA quality was determined using the Agilent 2100 Bioanalyzer (Agilent Technologies). RNA was then reverse-transcribed and expression of mouse eotaxin-1, eotaxin-2, IL-4R α , IL-13R α 1, TGF- β 1, and TIMP-1 mRNA analyzed using real-time quantitative PCR (TaqMan). The forward and reverse primers for mouse eotaxin-1, TIMP-1, and TGF- β 1 mRNA along with the FAM-5' end- and TAMRA-3' end-labeled fluorogenic probes were designed using Primer Express software (Mebio, McMaster University) as published previously (15, 53). Primers as well as FAM-5' end-labeled fluorogenic probes for mouse eotaxin-2, IL-4R α , and IL-13R α 1 mRNA and VIC-5' end-labeled fluorogenic probes for 18S RNA were obtained as gene expression assays from Applied Biosystems.

Sircol collagen assay

Analysis of total lung collagen content was performed using the Sircol Collagen Assay according to the manufacturer's instructions (Biocolor Life Science Assays, U.K.). Briefly, right mouse lungs were dissected and homogenized in cold PBS with 1 mg/ml aprotinin. Lung tissue homogenates were centrifuged at 10,000 rpm for 10 min at 4°C; supernatants were then collected and stored at –70°C for analysis. Supernatants (100 μ l) were mixed with 1000 μ l 0.1% Sirius Red Dye in saturated Picric Acid (Electron Microscopy Sciences) in microcentrifuge tubes for 30 min at room temperature. The collagen–Sirius Red Dye precipitate was compacted by centrifugation at 12,000 rpm for 10 min at room temperature. The unbound Sirius Red Dye was drained from microcentrifuge tubes and the bound dye released and dissolved by adding 1000 μ l 0.5 M sodium hydroxide. Released Sirius Red Dye was measured calorimetrically using a multiwell plate reader at a wavelength of 550 nm.

Cytokine and chemokine measurements

Mouse cytokine and chemokine protein expression in the BAL was measured using 15-plex immunoassay kits (Millipore) containing analytes for eotaxin-1 (minimum detection concentration [mdc] = 4.4 pg/ml), IFN- γ (mdc = 0.9 pg/ml), IL-12p40 (mdc = 4.9 pg/ml), IL-12p70 (mdc = 4.1 pg/ml), IL-13 (mdc = 6.3 pg/ml), IL-4 (mdc = 0.4 pg/ml), IL-5 (mdc = 0.5 pg/ml), IL-6 (mdc = 1.8 pg/ml), KC (mdc = 1.4 pg/ml), LIF (mdc = 0.8 pg/ml), and MCP-1 (mdc = 5.3 pg/ml). Mouse eotaxin-2 protein expression in the BAL was measured using an eotaxin-2 DuoSet ELISA kit purchased from R&D Systems (Minneapolis, MN). The detection limit of the eotaxin-2 DuoSet ELISA kit is 7.8 pg/ml (lowest standard point). ELISA assays were carried out according to the manufacturer's instructions.

Measurements of airway responsiveness

Parameters of airway responsiveness (airway resistance [Rn], tissue resistance/damping [G], and tissue elastance/stiffness [H]) were determined as published elsewhere (54). Briefly, mice were anesthetized with nebulized isoflurane, paralyzed with pancuronium bromide, tracheostomized, and mechanically ventilated with a small animal computer-controlled piston ventilator (flexi-Vent, SCIREQ). Mice received 200 breaths per minute and a tidal volume of 0.25 ml; the respiratory rate was slowed during nebulization (10 s) to provide

five large breaths of aerosol at a tidal volume of 0.8 ml. The response to nebulized saline and increasing doses (3.125, 12.5, and 50 mg/ml) of MCh (Sigma-Aldrich) was measured. A positive end expiratory pressure of 3 cm H₂O was applied by submerging the expiratory line in water. Respiratory impedance was determined from 3 s broadband volume perturbations ranging from 1–20.5 Hz every 10 s during ~2 min following each dose of MCh. The data were fitted with the constant phase model, and model parameters (Rn, G, H, and hysteresivity [η (η)]), a measure of lung heterogeneity [η = G/H]), were calculated. Model fits that resulted in a coefficient of determination <0.8 were excluded.

Results

Role of STAT6 in OSM-induced airway inflammation

To determine the role of STAT6 in the regulation of mOSM-induced cellular inflammation, WT and STAT6^{–/–} mice were treated with AdmOSM or AdDi70 and cell components in the BAL, lung tissue, and the blood compartment were assessed (Figs. 1, 2). In our previous study, we demonstrated a significant and sustained increase in airway eosinophils (days 7 and 14) in WT mice intranasally administered 5×10^7 PFU AdmOSM compared with control mice administered with AdDi70 (14). In the current study, Ad vectors

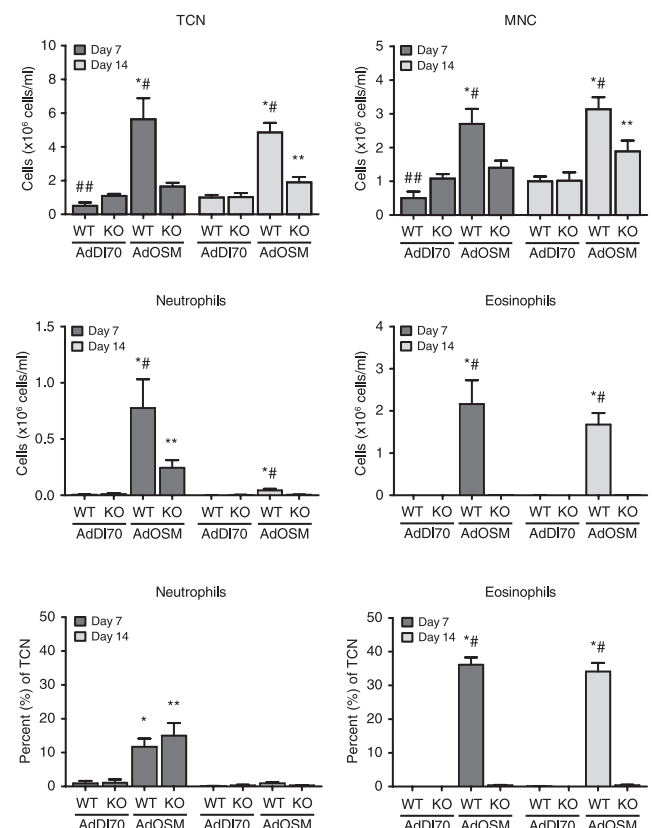


FIGURE 1. AdmOSM-induced airway eosinophilia is STAT6-dependent. WT or STAT6-knockout (KO) C57BL/6 mice were endotracheally administered with 5×10^7 PFU AdDi70 or AdmOSM and euthanized 7 or 14 d later. BAL fluid was obtained, cytopins prepared, and BAL cells counted as described in *Materials and Methods*. Differential cell analysis shows TCN, MNC, neutrophils, eosinophils, and the percentage of cells in BAL fluid of WT and KO mice administered with AdDi70 or AdmOSM. Data are expressed as mean $\pm$ SEM (n = 4–7 mice/AdDi70 group; n = 6 to 7 mice/AdmOSM group). Two-way ANOVA with Tukey post hoc test was used to test significant differences between groups of mice. Data were regarded as significant when p < 0.05 with *WT AdmOSM versus WT AdDi70, **KO AdmOSM versus KO AdDi70, and #WT AdmOSM versus KO AdmOSM. Data are representative of three separate experiments showing similar trends.

(AdmOSM and AdDI70) were administered through the intra-tracheal route to optimize pulmonary Ad vector delivery (5×10^7 PFU). AdmOSM-treated WT mice at day 7 developed a significant increase in the total cell number (TCN), MNC, neutrophils and eosinophils in the BAL compared with AdDI70-treated control mice (Fig. 1). Accumulation of MNC and eosinophils was sustained at day 14, and neutrophils were still evident, although at ~ 18 -fold less than observed at day 7 (Fig. 1). In contrast to WT mice, STAT6^{-/-} mice treated with AdmOSM showed significantly less airway MNC, neutrophils, and, most markedly, a complete inhibition of airway eosinophils (Fig. 1) at both time points examined. STAT6^{-/-} mice

displayed a modest elevation of TCN and MNC number upon AdDI70 treatment compared with WT mice.

To determine if eosinophils accumulate in the circulation and the lung parenchyma, we measured eosinophils in the blood and total lung by flow cytometry (Fig. 2). Flow cytometric measurements demonstrated a marked and significant influx of eosinophils into the whole lung of WT mice that was comparable in magnitude to the cellular responses observed in the BAL (Figs. 1, 2A). Consistent with the BAL data, AdmOSM-induced accumulation of lung tissue eosinophils was STAT6 dependent as demonstrated by marked abrogation of this leukocyte population in AdmOSM-

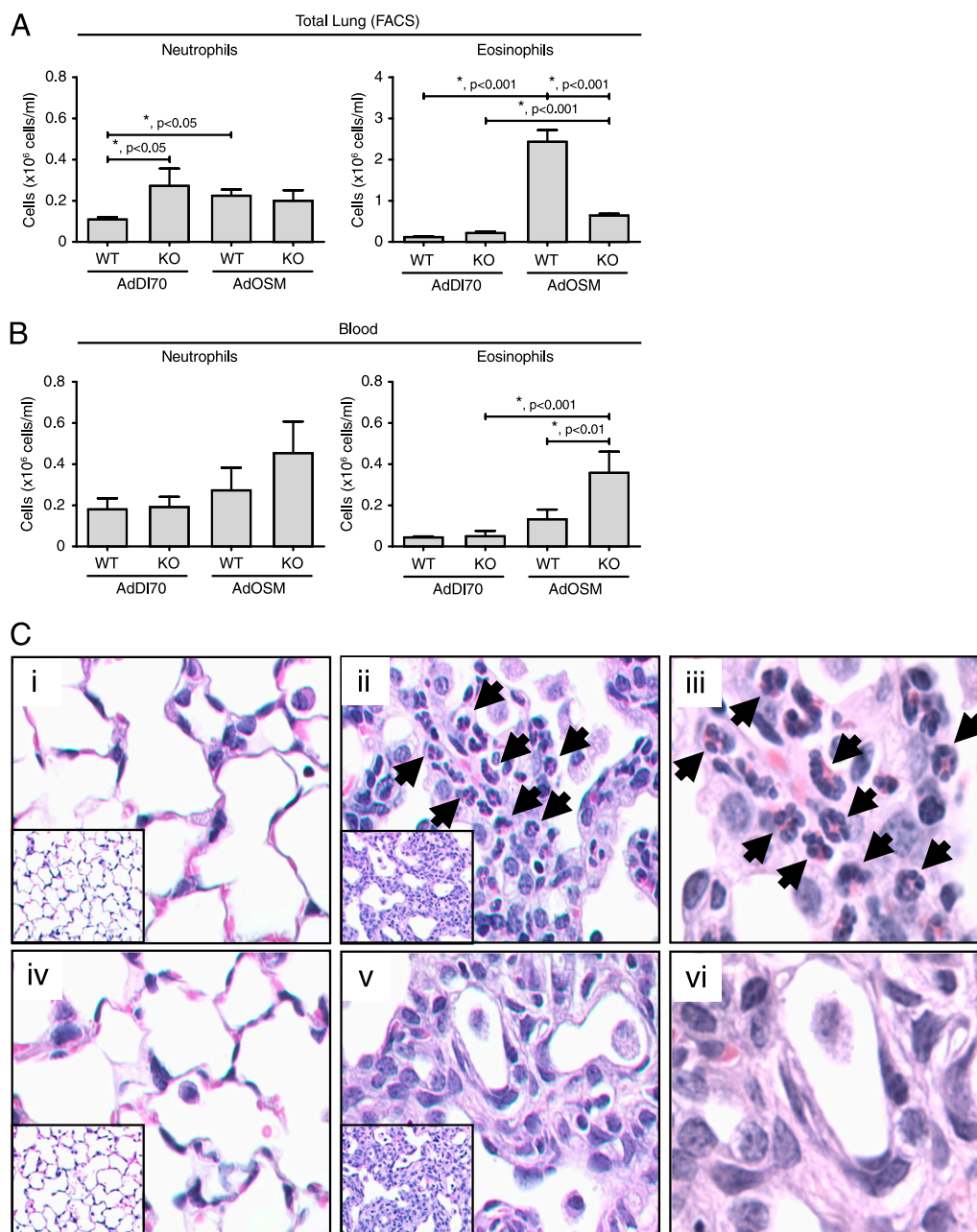


FIGURE 2. AdmOSM-induced tissue eosinophilia is STAT6 dependent. WT and STAT6-KO C57BL/6 mice were endotracheally administered with 5×10^7 PFU AdDI70 or AdmOSM and euthanized 14 d after. **A**, For lung tissue leukocyte analysis, cells were isolated from lungs and subjected to flow cytometry as described in *Materials and Methods*. **B**, For peripheral blood leukocyte analysis, blood was obtained, blood smears prepared, and cells counted as described in *Materials and Methods*. Data are expressed as mean $\pm$ SEM ($n = 9$ mice/AdDI70 group; $n = 9$ mice/AdmOSM group). Two-way ANOVA with Tukey post hoc test was used to test significant differences between groups of mice. **C**, Representative photomicrographs of paraffin-embedded cross-sections of lung tissues stained with H&E (insets, original magnification $\times 20$) are: WT mice treated with AdDI70 (original magnification $\times 40$) (**i**) or AdOSM at original magnification $\times 40$ (**ii**) and at original magnification $\times 80$ (**iii**); and STAT6^{-/-} mice treated with AdDI70 at original magnification $\times 40$ (**iv**) or AdOSM at original magnification $\times 40$ (**v**) and original magnification $\times 80$ (**vi**).

treated STAT6^{-/-} mouse lungs (Fig. 2A). Differential cell analysis in the blood compartment revealed a significant increase in blood eosinophils in AdmOSM-treated STAT6^{-/-} mice compared with AdmOSM-treated WT mice and AdDI70 control mice (Fig. 2B). Thus, lung tissue eosinophils and BAL eosinophils were dramatically reduced in STAT6^{-/-} mice despite an increase in blood eosinophils. In addition, a small and significant increase in lung tissue neutrophils was observed in WT mouse lungs administered AdmOSM (Fig. 2A). We also observed a trend toward an increase in

the number of blood neutrophils in AdmOSM-treated STAT6^{-/-} mice compared with similarly treated WT mice and AdDI70 control mice; however, this did not reach statistical significance (Fig. 2B).

Histological analysis of WT and STAT6^{-/-} mouse lungs is shown in Fig. 2C. Treatment of WT and STAT6^{-/-} mouse lungs with AdDI70 did not elicit any detectable increases in parenchymal inflammatory cell accumulation, and the parenchymal architecture appeared normal (Fig. 2Ci, 2Civ). Consistent with our previous findings (14), AdmOSM-treated WT mouse lungs developed

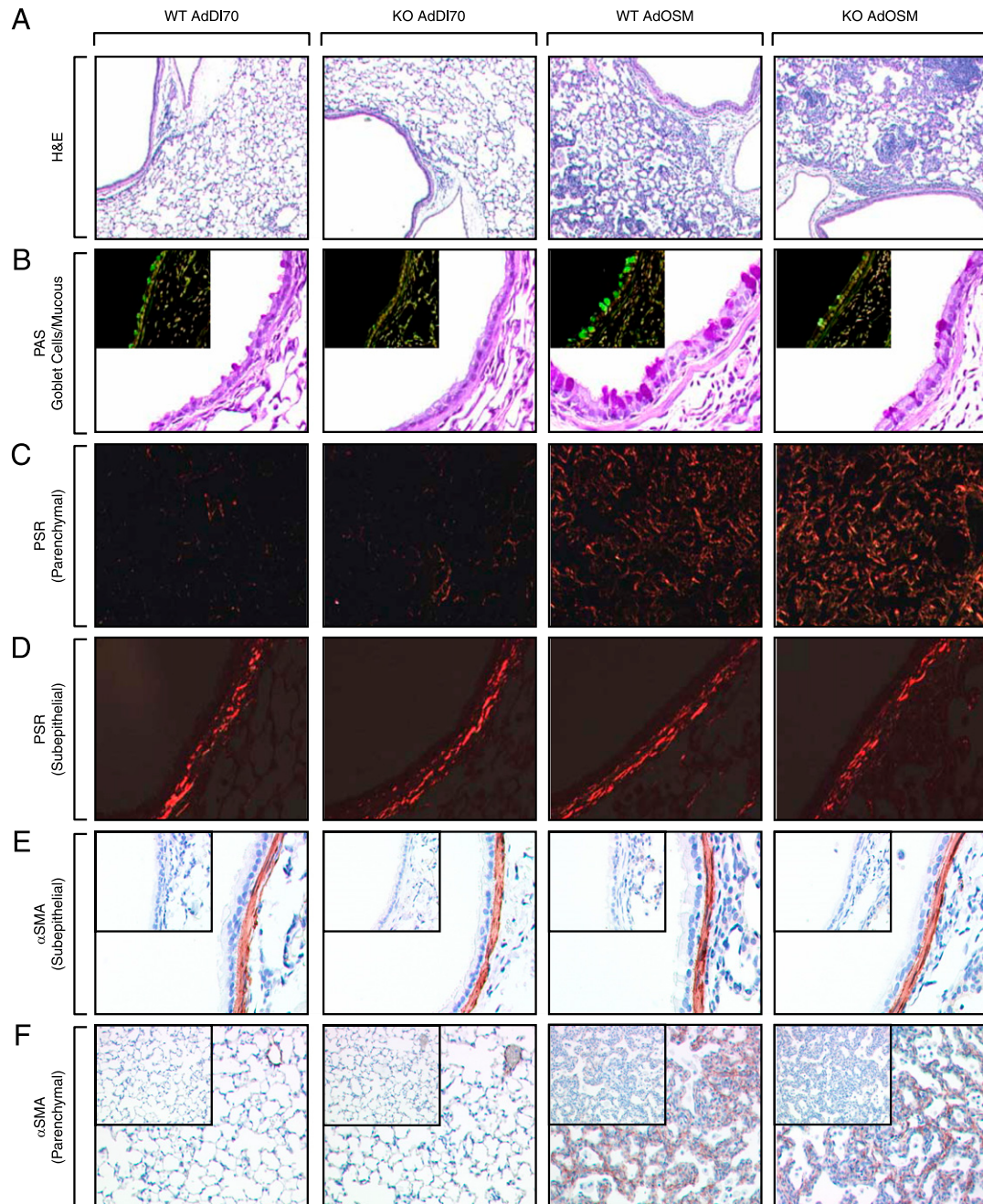


FIGURE 3. AdmOSM regulates pulmonary tissue remodeling independently of STAT6. WT or STAT6-KO C57BL/6 mice were endotracheally administered with 5×10^7 PFU AdDI70 or AdmOSM and euthanized 14 d later. Representative photomicrographs of paraffin-embedded cross-sections of lung tissues: H&E staining (middle portion of left lung, original magnification $\times 5$) (A), PAS staining denoting mucous production by epithelial goblet cells (main bronchus, middle portion of left lung, original magnification $\times 20$) (B) (magenta: insets depict color inverted image used for morphometric analysis). PSR staining denoting parenchymal collagen deposition (superior portion of the left lung, original magnification $\times 5$) (C) and subepithelial airway collagen deposition (main bronchus, middle portion of left lung, original magnification $\times 20$) (visualized under polarized light) (D). Representative photomicrographs of α SMA-immunostained paraffin-embedded cross sections of lung tissues denoting the major airway subepithelium (main bronchus, middle portion of left lung, original magnification $\times 20$) (E) and lung parenchyma (superior portion of left lung, original magnification $\times 10$) (F). Insets depict negative immunostaining with a control IgG.

inflammation characterized by diffuse infiltration of MNC, neutrophils, and eosinophils in the lung parenchyma. Eosinophils were seen in the intraseptal regions of alveolar walls (Fig. 2Cii, 2Ciii) and, to some extent, peribronchial and perivascular areas as published previously (14). In contrast, eosinophils were virtually absent in the lungs of AdmOSM-treated STAT6^{-/-} mice (Fig. 2Cv, 2Cvi). Alveolar septal hypertrophy appeared present in both AdmOSM-treated WT and STAT6^{-/-} mice. Similar results were seen at day 7 in histological analysis, in which very few eosinophils were present under high-power resolution, either in the alveolar space or the interstitium.

Role of STAT6 in OSM-induced airway and parenchymal remodeling

We next examined the effects of AdmOSM administration on mucous/goblet cell hyperplasia, collagen accumulation in the subepithelial layer, lung parenchyma, and total lung compartments (Fig. 3, quantification in Fig. 4) as well as smooth muscle actin and the requirement of STAT6 in regulation of these airway remodeling responses. Histological and morphometric analysis of PAS-stained sections of lungs derived from AdDI70-treated WT and STAT6^{-/-} mice at day 14 demonstrated presence of few mucin-staining PAS⁺ airway epithelial cells, whereas treatment of WT mouse lungs with AdmOSM induced significant and sustained increase in mucin-staining PAS⁺ cells in the airway epithelium (albeit levels at day 14 were slightly lower than at day 7; data not shown). In contrast, STAT6^{-/-} mouse lungs were protected from AdmOSM-induced increase in mucin/mucous producing airway epithelial cells (Figs. 3B, 4B), showing an essential role for STAT6 in mOSM-induced mucous/goblet cell hyperplasia.

Lung parenchymal ECM deposition was evident as previous (14); indeed, OSM has been implicated in interstitial pulmonary fibrosis in animal models (6, 13, 14) and clinical studies (6). We

therefore examined the role of STAT6 in AdmOSM-induced parenchymal collagen accumulation by histological and morphometric analysis of PSR-stained sections of mouse lungs (Figs. 3C, 4C). AdmOSM-treated WT and STAT6^{-/-} mouse lungs developed substantial and significant parenchymal collagen deposition in the interstitium evident at day 14. Because subepithelial membrane fibrosis is another characteristic of airway remodeling in asthma (55), we also examined collagen deposition in this locale in WT and STAT6^{-/-} mice (Figs. 3D, 4D). Analysis at day 14 (and at day 7, not shown) demonstrated that AdmOSM administration in WT mice, at this particular dose of Ad vector, had no detectable effect on subepithelial membrane collagen deposition above that observed in AdDI70-treated mice. When we examined total lung extracts at day 14 for collagen content by the Sircol assay, total collagen was markedly and comparably increased in both AdmOSM-treated WT and STAT6^{-/-} mice (Fig. 4A), corroborating the morphometric analysis of the lung parenchyma (Figs. 3C, 4C).

Thickening of airway smooth muscle can be another feature of airway remodeling in asthma (55), although it is not known whether the mOSM-STAT6 axis regulates this response in vivo. Therefore, we examined the effects of mOSM on airway smooth muscle thickness at day 14 by histological and morphometric analysis of lung sections immunostained for α SMA (Fig. 3E). We observed no apparent differences in the major airway smooth muscle between AdmOSM-treated WT mice and AdDI70-treated control mice, and comparable subepithelial α SMA⁺ immunostaining was observed between AdmOSM-treated STAT6^{-/-} and WT mice.

In contrast to the subepithelial compartment of the major airway, when examining parenchymal tissue, we observe a marked increase in α SMA⁺ immunostaining in the interstitium in both AdmOSM-treated WT and STAT6^{-/-} mice (Fig. 3F), which coincided with parenchymal collagen deposition. Results at day 7 show the same trends (data not shown) as outlined for day 14 in collagen, α SMA, and goblet cell accumulation, although the levels of goblet cells numbers in response to AdOSM were slightly higher than at day 14, and parenchymal collagen in STAT6^{-/-} mice treated with AdOSM was slightly elevated compared with WT mice treated with AdOSM. These data further support the interpretation that STAT6 is not required for parenchymal collagen or α SMA accumulation in this system. Collectively, these findings implicate OSM in STAT6-independent regulation of parenchymal ECM accumulation.

Role of STAT6 in OSM-induced pulmonary cytokine and chemokine expression

Using immunoassay profiling, we measured cytokine and chemokine protein levels from BAL to explore the mechanism(s) associated with mOSM action in regulating inflammatory and tissue remodeling responses (Fig. 5). Treatment of WT mouse lungs with AdmOSM induced significant increases in IL-4 (~32-fold), IL-5 (~32-fold), IL-13 (~2-fold), MCP-1 (~2-fold), KC (~9-fold), eotaxin-1 (~2-fold), and eotaxin-2 (~200-fold) during the early phase of the inflammatory response (day 7) (Fig. 5). Induction of these mediators was largely abolished in STAT6^{-/-} mice with the exception of IL-5 and KC (Fig. 5). Levels of IL-4, MCP-1, KC, and eotaxin-2 remained elevated in the BAL of WT mouse lungs 14 d following AdmOSM treatment (although somewhat reduced from day 7 levels), and BAL levels were ablated in similarly treated STAT6^{-/-} mice (Fig. 6). Therefore, MCP-1 and eotaxin-2 induction appear to correlate with infiltration of MNC and eosinophils, respectively, in AdmOSM-treated mouse lungs. We also examined levels of typical Th1 cytokines (IL-12, IFN- γ) and found very small differences between different groups of mice (<15%) that were considered negligible in comparison with the Th2 cytokines. This

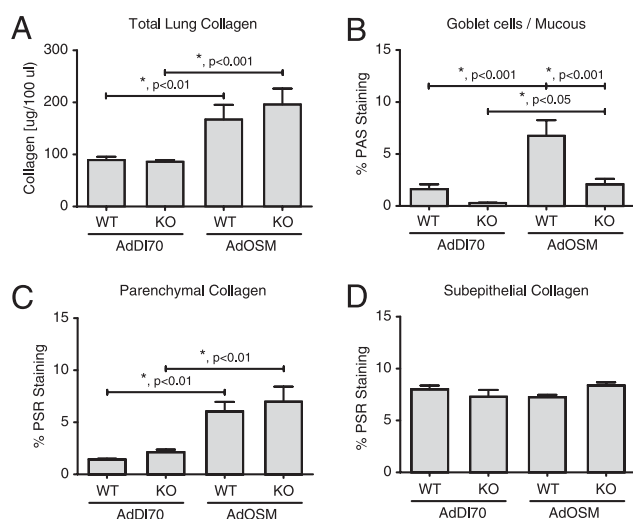


FIGURE 4. Quantification of collagen deposition and goblet cells. WT or STAT6 KO mice were treated as in Fig. 1 ($n = 4-7$ per group), and lung extracts or histologically stained samples were assessed quantitatively. A, Total collagen was measured in samples of total lung extracts by Sircol assay as in *Materials and Methods*. Morphometric analysis of photomicrographs, such as those shown in Fig. 3, were completed of PAS-stained (B) and PSR-stained paraffin-embedded cross-sections of lung tissue for parenchymal collagen staining (C) and subepithelium (D). Data are expressed as mean $\pm$ SEM ($n = 4-7$ mice/AdDI70 group; $n = 6$ to 7 mice/AdOSM group). Two-way ANOVA with Tukey post hoc test was used to test significant differences between groups of mice (differences and p values are indicated). Data are representative of three separate experiments showing similar trends.

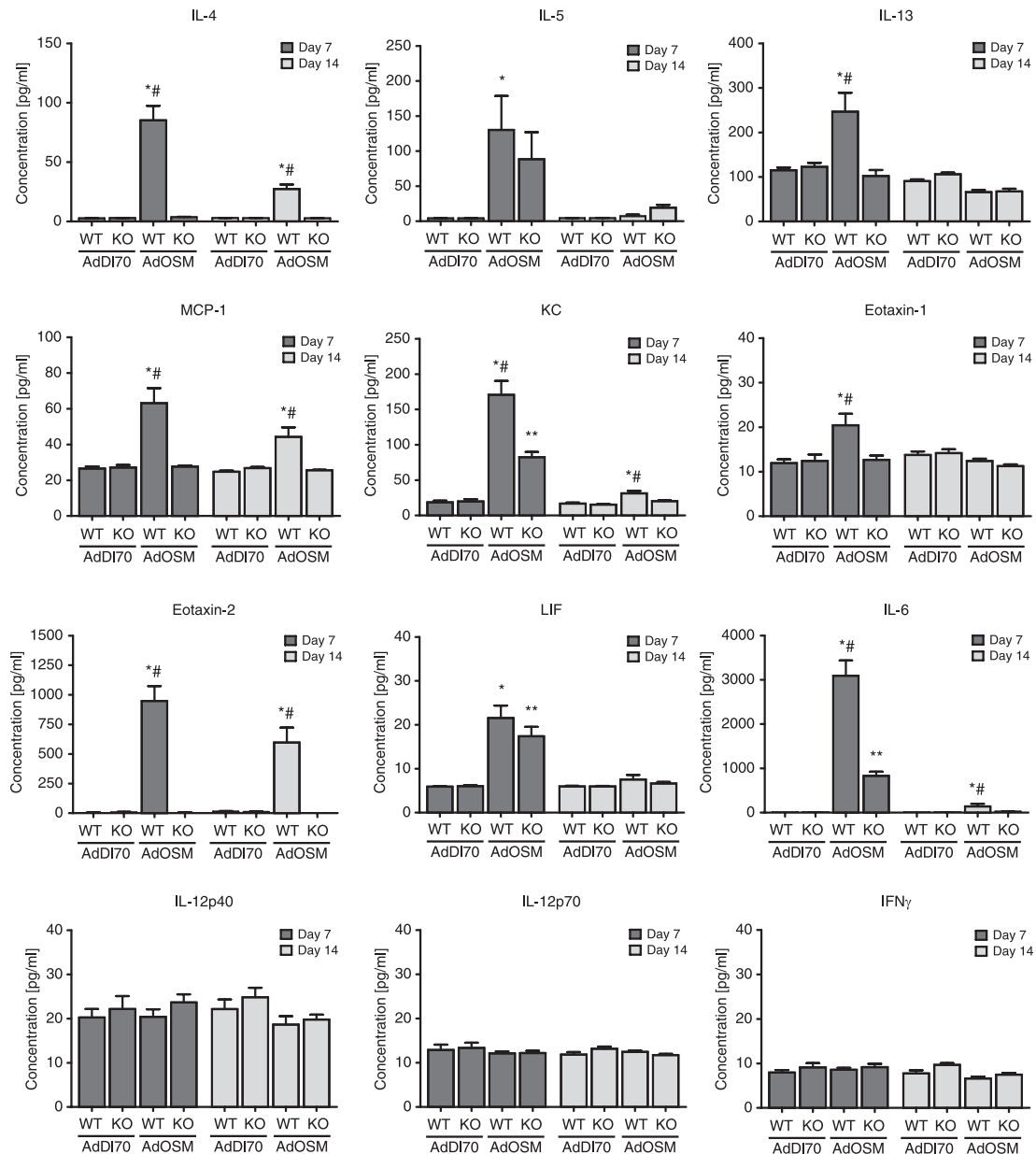


FIGURE 5. AdmOSM induces chemokines and Th2 cytokines. WT or STAT6-KO C57BL/6 mice were endotracheally administered with 5×10^7 PFU AdDI70 or AdmOSM and euthanized 7 or 14 d later. BAL fluid was obtained and differential BAL protein measured by 15-PLEX analyte system (LUMINEX) or ELISA as described in *Materials and Methods*. Data are expressed as mean $\pm$ SEM ($n = 4-7$ mice/AdDI70 group; $n = 6$ to 7 mice/AdmOSM group). Two-way ANOVA with Tukey post hoc test was used to test significant differences between groups of mice. BAL protein levels were regarded as significant when $p < 0.05$ with *WT AdmOSM versus WT AdDI70, **KO AdmOSM versus KO AdDI70, and #WT AdmOSM versus KO AdmOSM. Data are pooled from two separate experiments.

indicates that AdmOSM-induced lung pathology was primarily associated with a predominant Th2 inflammatory profile. We also detected significant induction of the gp130 cytokines LIF (~ 4 -fold) and IL-6 (~ 480 -fold) in the BAL from WT mice treated with AdmOSM, and only IL-6 levels were significantly reduced (not abolished) in STAT6 $^{-/-}$ mice (Fig. 5).

Whole-lung RNA analysis for several genes revealed consistency with that predicted from BAL protein analysis. AdmOSM-treated WT mouse lungs showed significant and sustained increase in eotaxin-1 and eotaxin-2 mRNA levels that were abolished in similarly treated STAT6 $^{-/-}$ mouse lungs (Fig. 6). Investigation of the IL-4R chain components demonstrated significantly elevated although transient expression of whole-lung IL-4R α mRNA in AdmOSM-treated WT and STAT6 $^{-/-}$ mice compared with their

respective AdDI70 control mice, indicating STAT6-independent regulation of IL-4R α by mOSM in vivo (Fig. 6).

Role of STAT6 in OSM-induced TIMP and MMP and collagen mRNA expression

We also examined genes implicated in ECM remodeling. Analysis of whole-lung TGF- β mRNA revealed negligible changes in basal levels between different groups of mice as observed 14 d following Ad vector administration (Fig. 6), suggesting that TGF- β may not be involved in AdmOSM-induced parenchymal interstitial fibrosis. In addition, treatment of WT mouse lungs with AdmOSM induced a significant albeit transient increase in whole-lung TIMP-1 mRNA levels, which were significantly reduced (not abolished) in AdmOSM-treated STAT6 $^{-/-}$ mouse lungs (Fig. 6).

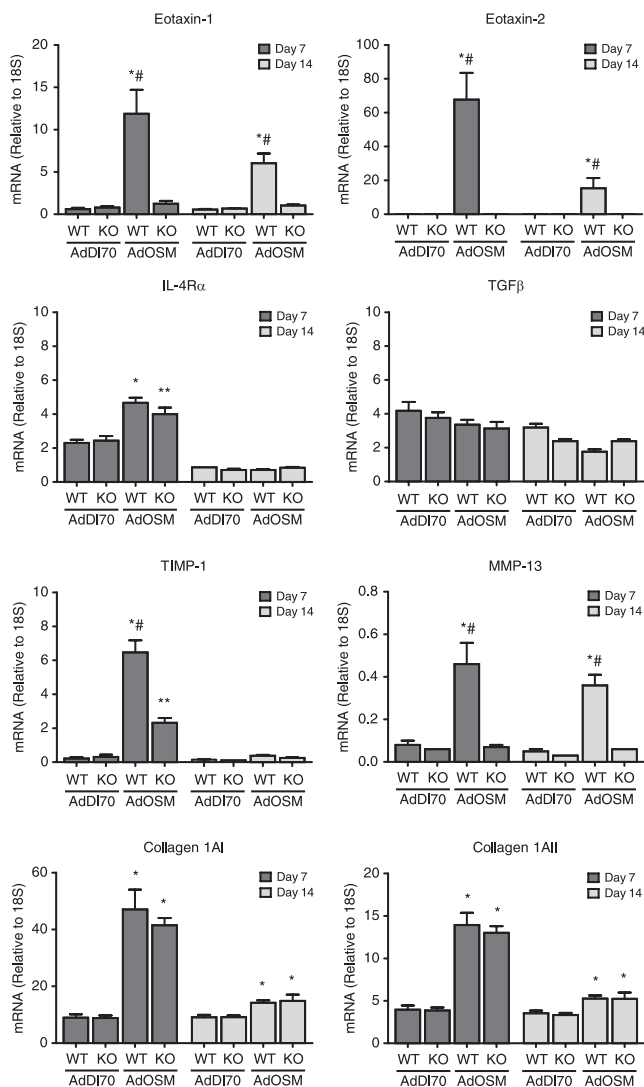


FIGURE 6. AdmOSM induces total lung eotaxin-1/2, IL-4R, TIMP-1, and MMP-9 mRNA expression. WT or STAT6-KO C57BL/6 mice were endotracheally administered with 5×10^7 PFU AdDI70 or AdmOSM and euthanized 7 or 14 d later. Lungs were homogenized in TRIzol and RNA extracted and quantitated using RT-PCR (TaqMan) as described in *Materials and Methods*. Data are expressed as mean $\pm$ SEM ($n = 4-7$ mice/AdDI70 group; $n = 6$ to 7 mice/AdmOSM group). Two-way ANOVA with Tukey post hoc test was used to test significant differences between groups of mice. mRNA transcript levels were regarded as significant when $p < 0.05$ with *WT AdmOSM versus WT AdDI70, **KO AdmOSM versus KO AdDI70, and #WT AdmOSM versus KO AdmOSM. Data are representative of two separate experiments showing similar trends.

Whole-lung mRNA analysis of MMPs revealed that AdmOSM elicited a marked and sustained induction in MMP-13 mRNA levels in a STAT6-dependent manner. When we assessed levels of collagen 1A1 and 1A2 mRNA, we observed marked elevation of both mRNA species at day 7 (~ 5 -fold), which was reduced but not absent at day 14 (Fig. 6). STAT6 $^{-/-}$ mice responded in very similar manner, indicating that coll 1A1 and 1A2 mRNA was regulated independently of STAT-6 in this model.

Role of OSM-STAT6 axis on lung function

To determine if AdmOSM causes changes in lung physiology, we examined lung function using the flexiVent system and increasing doses of nebulized MCh in WT and STAT6 $^{-/-}$ mice 14 d following AdmOSM or AdDI70 administration. The peak response at each

MCh dose (maximal response) and the slope of the dose-response relationships (reactivity) were calculated (Fig. 7). We did not observe any significant differences in the baseline lung function conditions among different groups of mice (data not shown). Treatment of WT mouse lungs with AdmOSM induced a significant increase in airway reactivity (Fig. 7, *top right panel*) that was reduced in STAT6 $^{-/-}$ mouse lungs, although this reduction did not reach statistical significance. Administration of AdmOSM into WT mouse lungs also caused a significant increase in both tissue resistance (damping) and tissue elastance (stiffness) in response to MCh compared with AdDI70-treated groups of mice (Fig. 7). Such increased maximal responsiveness and reactivity in the lung tissue compartment was completely abolished in AdmOSM-administered STAT6 $^{-/-}$ mice (Fig. 7). Because hysteresivity ($\eta = G/H$) was relatively homogenous (unchanged) between different groups of mice (data not shown), this indicates that the increases in tissue resistance and tissue elastance observed in AdmOSM-treated WT mice are likely associated with airway narrowing/closure, which leads to derecruitment of the alveolar units.

Discussion

Our experimental work presented in this study demonstrates the novel finding that administration of AdmOSM into mouse lungs induces a predominant Th2 inflammatory profile that requires func-

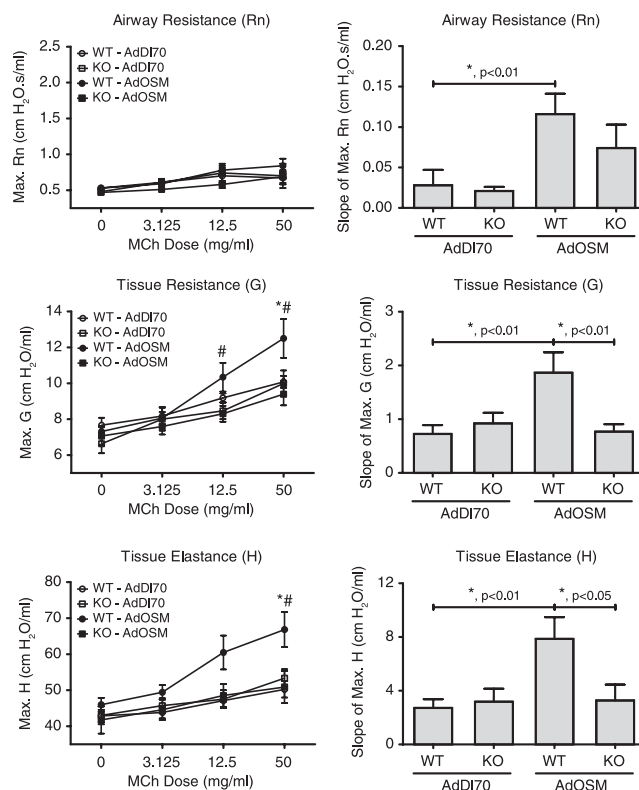


FIGURE 7. AdmOSM induces AHR. Analysis of airway responsiveness to MCh in WT or STAT6-KO C57BL/6 mice 14 d following endotracheal administration of 5×10^7 PFU AdDI70 or AdmOSM. Rn, G, H, and hysteresivity (η) were measured using a flexiVent system in response to increasing doses of nebulized MCh (0, 3.125, 12.5, and 50 mg/ml). Data are expressed as mean $\pm$ SEM ($n = 8-10$ mice/group). Two-way ANOVA with Tukey post hoc test was used to test significant differences between groups of mice. *Left panels*, MCh dose-response relationships showing maximal (peak) responses were regarded as significant when $p < 0.05$ with *WT AdmOSM versus WT AdDI70 and #WT AdmOSM versus KO AdmOSM. *Right panels*, Slopes of MCh dose-response relationships of maximal responses show reactivity.

tional STAT6 signaling; however, induction of matrix remodeling was STAT6 independent. To our knowledge, these results are also the first to show that overexpression of OSM causes changes in mouse lung physiology characterized by increased airway reactivity, tissue resistance (damping), and tissue elastance (stiffness). Changes in AHR were dependent on STAT6 and correlated with pulmonary eosinophil accumulation. The STAT6 signaling/transcription factor was essential for the induction of the Th2 cytokines IL-4 and IL-13, chemokines (MCP-1, KC, eotaxin-1/2), and pulmonary eosinophilia, but not for induction of IL-5 and blood eosinophilia. Absence of eosinophils in STAT6^{-/-} mouse lungs occurred despite elevated IL-5 and blood eosinophils. Overexpression of mOSM in mouse lungs also elicited marked mucous/goblet cell hyperplasia in a STAT6-dependent manner that paralleled the infiltration of mouse lungs with eosinophils. In addition, AdmOSM-treated mouse lungs developed marked collagen deposition and α SMA accumulation in the interstitium of the lung parenchyma independently of STAT6 and independently of elevation of IL-4/IL-13, neutrophils, and eosinophils.

AHR is a defining feature of human asthma (55) and is characterized by increased sensitivity of the pulmonary airways to a bronchoconstrictor resulting in a shift to the left of the bronchoconstrictor dose-response relationship (hypersensitivity), a steeper slope of the bronchoconstrictor dose-response relationship (hyperreactivity), and a greater maximal response to the bronchoconstrictor. The observed AHR in AdmOSM-administered WT compared with STAT6^{-/-} mouse lungs may be associated with the extent of cellular inflammation and/or the effects of mediators released by the inflammatory cells. The pronounced inflammatory cell accumulation observed in AdmOSM-treated WT mouse lungs may lead to AHR by causing airway narrowing/closure, resulting in derecruitment of alveolar units. The eosinophilic infiltration of mouse lungs that follows aeroantigen provocation has been associated with the development of AHR in some animal models of acute allergic airway inflammation (56–58). This is consistent with the observations made in our *in vivo* model system. The release of various mediators by inflammatory cells, including the Th2 cytokines IL-4 and IL-13, can also contribute to the development of AHR (22–26). Because both IL-4 and IL-13 signal through the IL-4R α –STAT6 pathway (30, 31), it is evident that STAT6^{-/-} mice are protected from aeroantigen-induced AHR (32–34). However, it is possible that OSM may regulate lung physiology directly through STAT6 (15) and independently of IL-4/IL-13 in our animal model, although further investigations are required. The inability of AdmOSM to induce significant increases in maximum Rn (Fig. 7, *top panel*) may be a consequence of the mouse strain used because C57BL/6 mice are known to have lower responses to MCh challenge compared with BALB/c mice (59).

Mucous/goblet cell hyperplasia is a common characteristic of the airway remodeling response in asthmatics (55). OSM has been recently shown by Mozaffarian et al. (6) to increase mucin/mucous containing airway epithelial cells; however, the potential mechanisms involved in this response have not been elucidated. Experimental models of acute allergic airway inflammation have shown that STAT6 is an indispensable regulator of aeroantigen-induced mucous hypersecretion (32, 33). Our studies confirm that mOSM elicits marked mucous/goblet cell hyperplasia and demonstrate further that STAT6 is an essential mechanistic component in regulation of this airway remodeling response. IL-4 and IL-13 have been shown to directly stimulate mucous/goblet cell hyperplasia (22–24), and in this study, we demonstrate that AdmOSM-treated mouse lungs have a significant and STAT6-dependent increase in both IL-4 and IL-13 production, implicating these Th2 cytokines in regulation of the goblet cell phenotype in our animal

model. Alternatively, OSM or other factors may have a direct effect on mucin/mucous expression in the airway epithelium; however, further experimental work is required to determine such possibilities. Several animal studies of asthma utilizing selective eosinophil-depletion strategies have linked this leukocyte population with the development of mucous/goblet cell hyperplasia (56, 57); however, others have not (58). In this study, we note that AdmOSM-induced sustained increase in mucin/mucous positive airway epithelial cells was associated with persistent accumulation of eosinophils in mouse lungs, suggesting that eosinophil-released inflammatory mediators may play a role. However, we cannot exclude the role of other leukocytes in contributing to the mucous/goblet cell phenotype.

Recent clinical studies have shown increased levels of OSM protein in the BAL fluid from subjects with IPF (6) or scleroderma-associated interstitial lung disease (60), suggesting that OSM may be involved in interstitial pulmonary fibrosis. Our previous studies have demonstrated that transient overexpression of OSM in mouse lungs has pronounced effects on pulmonary ECM remodeling (13, 14). These findings have been recently extended by Mozaffarian et al. (6), who demonstrated that OSM-induced total lung collagen accumulation is independent of IL-4/IL-13 and TGF- β because pharmacological neutralization of these potential fibrogenic mediators had no significant impact on whole-lung collagen deposition. Our data support these findings because IL-4/IL-13 as well as TGF- β expression in AdmOSM-treated mouse lungs did not correlate with the development of interstitial pulmonary fibrosis. The observations of total lung collagen (Fig. 4A) and increases in collagen as assessed by PSR staining in parenchyma (Figs. 3C, 4C) indicate that overexpression of mOSM causes interstitial pulmonary fibrosis marked by parenchymal collagen deposition and increased α SMA (Fig. 3F) accumulation. Taken together, these data indicate that mOSM-induced parenchymal ECM deposition does not require STAT6 and suggest that the greatest changes in collagen content occur in the lung parenchyma in this model. We did not detect remodeling of the central conducting airway subepithelium (Figs. 3C, 4). ECM accumulation in subepithelium compartments of smaller airways is possible (our own observations), however technically difficult to quantify, and higher or longer OSM expression may induce more pronounced effects on the major airways. Because OSM activates STAT6 (15) and because this signaling/transcription factor has been associated with the development of tissue fibrosis in some *in vivo* model systems (18), we hypothesized that OSM may regulate parenchymal ECM deposition through this pathway. Our results show that STAT6 is not required for the mOSM-induced parenchymal collagen or α SMA deposition that indicates myofibroblast hyperplasia. These findings extend those shown previously by Mozaffarian et al. (6) by demonstrating that parenchymal ECM deposition induced by mOSM involves a STAT6-independent process. Parenchymal ECM accumulation may be associated with OSM direct actions on fibroblast collagen production. Our results support the possibility of direct action on collagen mRNA (1A1 and 1A2) and total lung collagen protein (Figs. 3, 4, 6) through a STAT6-independent mechanism. OSM has previously been shown to activate collagen transcription *in vitro* involving SP1 transcription factors (61). Further analysis is required to determine if other indirect pathways invoked by OSM are involved. For example, OSM may promote epithelial–myofibroblast transdifferentiation characterized by α SMA and fibronectin expression, as has been documented in kidney proximal tubular epithelial cells (62).

Eosinophil migration into aeroantigen-provoked mouse lungs during an acute allergic airway inflammation is dependent on STAT6 (32–34). In this study, we demonstrate an essential role of

STAT6 in regulation of lung eosinophil accumulation in response to mOSM because STAT6^{-/-} mice were protected from AdmOSM-induced pulmonary eosinophilia. The sustained eosinophil accumulation correlated with eotaxin-2 expression, suggesting that this eosinophil selective CC chemokine mediates pulmonary eosinophilia in this in vivo model system. OSM has been shown to directly regulate eotaxin-1 induction in lung fibroblasts and smooth muscle cells (14–16). Whether OSM is able to directly regulate eotaxin-2 expression in lung structural cells is currently not known. In addition, mOSM-induced pulmonary accumulation of eosinophils corresponded with accumulation of MNC (macrophages/monocytes, lymphocytes). Macrophages have been shown to be the predominant source of eotaxin-2 expression in mouse lungs exposed to an acute aeroantigen provocation protocol (63), suggesting that sustained recruitment of eotaxin-2-producing macrophages into mOSM-treated mouse lungs may mediate the sustained pulmonary eosinophilia in our in vivo system. We observed a modest increase in MNC in AdD170-treated STAT6^{-/-} mice compared with WT mice at day 7 (Fig. 1), which may be associated with an increase in bone marrow-committed myeloid progenitor cells, a phenotype that is characteristic to this STAT6^{-/-} strain (64, 65). In addition, recent studies by Mozafarian et al. (6) demonstrated that RAG2^{-/-} mice failed to develop OSM-induced pulmonary eosinophilia, indicating that recruitment of lymphocytes is a necessary prerequisite for orchestrating the infiltration of eosinophils into mouse lungs treated with OSM. This parallels acute allergic airway disease models in which CD4⁺ T lymphocytes are essential in the establishment of aeroantigen-induced eosinophilic inflammation (66).

Examination of eosinophils in the peripheral blood during the later stage of AdmOSM-induced pulmonary inflammation (day 14) revealed a significant increase in blood eosinophils in STAT6^{-/-} mice, indicating possible involvement of STAT6-sensitive adhesion mechanisms in pulmonary eosinophil accumulation. Studies have shown that P-selectin and VCAM-1, which are upregulated on activated vascular endothelium, mediate tethering and firm arrest of eosinophils through P-selectin glycoprotein ligand 1 and $\alpha 4\beta 1$ (VLA-4) integrin interactions, respectively (67–70). Upregulation of P-selectin and VCAM-1 has been documented following OSM (71, 72) or IL-4 and IL-13 (73–75) stimulation of vascular endothelial cells. Because STAT6 is necessary for P-selectin (73, 74), but not VCAM-1 (15, 32, 76) expression, this suggests that abrogation of eosinophil accumulation into STAT6^{-/-} mouse lungs following AdmOSM administration could be attributed to the inhibition of P-selectin upregulation in concert with abolished eotaxin-1/2 expression. Neutrophils also use P-selectin glycoprotein ligand 1–P-selectin interactions during cell trafficking (68), and their chemotaxis is mediated by the neutrophil-selective CXC chemokine KC (77). Therefore, the resulting increase in neutrophils in the peripheral blood and their absence in the airways of AdmOSM-treated STAT6^{-/-} mice may also reflect changes in P-selectin and KC expression. However, further studies are required to examine the potential role of adhesion molecules in these observations.

Among members of the gp130 cytokines, OSM has a number of novel biological activities, particularly in regulation of connective tissue cells (9, 14, 15), and its role in lung inflammation in vivo may also be distinct. We do observe a marked increase in IL-6 (and very modest increase in LIF) protein levels in the BAL from AdmOSM-treated mouse lungs during the early phase of lung inflammation (Fig. 4). In other systems, transgenic overexpression of IL-6 elicited peribronchial MNC lymphoid aggregates and airway wall thickening associated with subepithelial fibrosis; however, it did not induce pulmonary eosinophilia, and mice were hyporesponsive in methacholine-induced airway response assess-

ment (78–80). The hyporesponsive nature of IL-6-transgenic mouse lungs was associated with a proportional increase in the airway caliber to the airway wall thickness (81). Because the pathological features observed in IL-6-transgenic mice contrast those observed in our in vivo model system, we suggest that the observed pulmonary inflammation, remodeling, and changes in lung physiology imparted by OSM are not by IL-6 directly or indirectly. Together, these results support a novel role of OSM in airway inflammation and that OSM regulates a set of STAT6-dependent inflammatory and STAT6-independent tissue remodeling responses that may parallel mechanisms found in both human asthma and IPF. This supports the concept of this gp130 cytokine as a potential therapeutic target in certain pulmonary disorders.

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Disclosures

The authors have no financial conflicts of interest.

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Supplementary material

Supplementary Figure-1. FACS gating strategy for isolating lung tissue neutrophils and eosinophils.

Single cell suspensions of whole lungs were generated from mice treated with AdOSM or AdDI70 as described in methods. The figure shows a schematic representation of the strategy using anti-CD45 and anti-GR-1.

Supplementary Figure-1

