Online Supplementary Material

Methodology

Subject Recruitment

Twenty-seven subjects were recruited from the general Research Triangle, North Carolina community. The asthmatics fulfilled criteria for asthma by exhibiting a provocative concentration of methacholine resulting in a 20% fall in the forced expiratory volume in one second (PC\textsubscript{20} FEV\textsubscript{1}) of < 8 mg/ml and reversibility, as demonstrated by at least a 12% and 200 ml increase in the FEV\textsubscript{1} or the forced vital capacity (FVC) with inhaled albuterol. Asthmatic subjects used short acting β-2 agonists only and normal subjects took no medications, demonstrated a PC\textsubscript{20} FEV\textsubscript{1} > 25 mg/ml and had no clinical history of atopy. Exclusion criteria included post-bronchodilator FEV\textsubscript{1} < 50% predicted, inpatient status, upper or lower respiratory tract infection within one month of study, use of anti-inflammatory controller medications within four weeks of study, long acting beta-2 agonists within two weeks of study, smoking history greater than 5 pack-years or any cigarette use within the previous two years, significant non-asthma pulmonary disease or other medical problems. All subjects provided informed consent in this Duke Institutional Review Board-approved protocol.

Airway Fibroblast Culture

Endobronchial tissue sections were placed on collagen-coated plates and fibroblasts were cultured. As numerous passages can induce cellular metaplasia and senescence, only cells from the first three passages were used for experimentation. Normal and asthmatic fibroblasts were cultured and passaged under identical conditions.

Mediator Exposure

When the cells were at confluence for seven days, media was changed, and cells were incubated in serum-free media for 24 hours. Cells were subsequently treated with and without
IL-13 (ProSpec, Rehovot, Israel; 50ng/ml) for 48 hours. Serum Free Media was used as control. In addition, airway fibroblasts were treated with IL-13 (50 ng/ml) and the pan-MMP inhibitor GM6001 (10 µM), (Galardin, Calbiochem, San Diego, CA), either alone, or in combination, which were also added in triplicate and incubated for 48 hours. GM 6001 inhibits MMP-1 (fibroblast collagenase), MMP-2 (gelatinase A), MMP-3 (stromelysin-1), MMP-8 (neutrophil collagenase) and MMP-9 (gelatinase B).

Airway fibroblasts were also exposed to specific MMP inhibitors, alone or in combination with IL-13, including inhibitors to MMP-1 (20 µM), MMP-2 (40 µM), and MMP-3 (20 µM) each alone and in combination with IL-13 (MMP1-Inhibitor, MMP2-Inhibitor-2, MMP3-Inhibitor; Calbiochem, San Diego, CA).

Lastly, airway fibroblasts were incubated in the presence of a TGFβRII neutralizing antibody at its ND50 dose (20 μg/ml) or a polyclonal goat IgG control antibody (R&D, Minneapolis, MN) alone or with IL-13 (50 ng/ml) for 48 hours. Concentrations of mediators were chosen based on dose response experiments (data not shown).

**Quantitative Real-time RT-PCR**

Total RNA from airway fibroblasts was extracted using Trizol after exposure to mediators. Collagen type 1 α-1 (COL1A1), collagen type 1 α-2 (COL1A2), collagen type 3 α-1 (COL3A1), matrix metalloproteinase-2 (MMP2) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers were purchased from Applied Biosystems (Hs01028956_m1, Hs01076777_m1, Hs00943793_m1, Hs00234422_m1, Hs99999905_m1, respectively). Relative mRNA expression was calculated by normalization of all expression levels to GAPDH and then compared to untreated control cells by the ΔΔCT method.
MMP-2 Activation Assay

Supernatants from cultured airway fibroblasts were collected at 48 hours with and without IL-13 (50 ng/ml) treatment in asthmatic and normal subjects. Measurement of MMP-2 activity was performed by using a commercial activity assay system according to manufacturer’s protocol (GE Healthcare, Buckinghamshire, UK). Briefly, supernatants were centrifuged to remove particulate matter. All reagents and working standards were prepared according to protocol. Standards and samples were incubated overnight at 4°C. Since endogenous MMP-2 activity was measured, p-aminophenylmercuric acetate (APMA) solution was not used. Assay buffer and detection reagent was placed into all wells. The plate was read to calculate mean absorbance at 405nm at t₀ and t₆ hour incubation period. Results are reported as a ratio of the mean absorbance₄₀₅ of IL-13-treated cells to untreated fibroblasts.

Luciferase Activity

CCL64 mink lung epithelial cell line was exposed for 24 hours to supernatants from either untreated or IL-13 (50 ng/ml) treated fibroblasts. Cells were lysed and luminescence developed according to instructions for a high sensitivity luciferase kit (Perkin Elmer, Waltham, MA). Luciferase expression was measured using a luminometer (BMG Labtech, Ortenberg, Germany) and results were expressed as relative luciferase units (RLU).

Western Blot Analysis

Cell lysates from cultured airway fibroblasts were collected at 48 hours after IL-13 (50 ng/ml) treatment. Cell lysates were resolved on 10% SDS-PAGE, and transferred to a PVDF membrane. The membrane was probed with rabbit polyclonal anti-human α-SMA antibody
(Abcam, Cambridge, MA). The membrane was stripped using western blot stripping buffer (Fisher, Rockford, IL) and re-probed using rabbit anti-human desmin antibody or rabbit anti-human GAPDH antibody (Abcam, Cambridge, MA). The blot was developed by enhanced chemiluminescence, documented with the Kodak Image Station 4000 MM PRO and quantified using Carestream Molecular Image software (version 5.0).
**Figure Legends**

**Figure S1.** Collagen type-1 protein expression as measured by ELISA in untreated airway fibroblasts in asthma (n=11) and normal controls (n=8). Although no significant difference was seen between the group means at baseline, there was individual variability within each group. Therefore, data in this study are presented as ratios to untreated fibroblasts.