The role of Interleukin-1 in driving inflammation and remodeling in the asthmatic-EMTU

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a place of mind

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Introduction

Asthma is a chronic inflammatory disease of the airways that is associated with airway remodeling, which involves all tissues of the airway wall including the epithelium, sub-mucosal, smooth muscle layers and vascular structures. In particular, a well documented feature of airway remodeling in asthma, is the accumulation of fibroblasts / myofibroblasts in the epithelial-mesenchymal trophic unit (EMTU) that leads to the deposition of excess collagen in the laminar propia of the airways. Our previous work has shown that airway epithelial cells, through the production of IL1α, regulate the fibroblasts within the lung EMTU[1].

Specific Aim

The aim of this study was to assess the effects of epithelialderived IL1 on fibroblastinduced inflammation, ECM production and remodeling within the asthmatic EMTU.

Methods & Materials

Primary airway epithelial cells (PAECs) and primary airway fibroblasts (PAFs) were obtained from both asthmatic and non-asthmatic donor lungs deemed not suitable for transplantation through the International Institute for the Advancement of Medicine.

PAECs were cultured at airliquid interface (ALI) for 20 days. RNA & supernatant were harvested at days 0, 5, 11 & 20 of ALI culture & the expression and release of IL1α and other IL1 family members was determined by RNA sequencing and ELISA respectively.

PAFs were seeded on collagen I gels stimulated with 1ng/ml IL-1α, IL-1β , IL-33 or media control for24 hrs as described below in figure 1 .

PAFs were also seeded on collagen I coated plates and stimulated with 1ng/ml IL-1α, IL-1β, IL-33 or media control for 24 hrs. RNA was harvested for qRT-PCR and the release of pro-inflammatory mediators was measured using ELISA.

Collagen I gel contraction assay

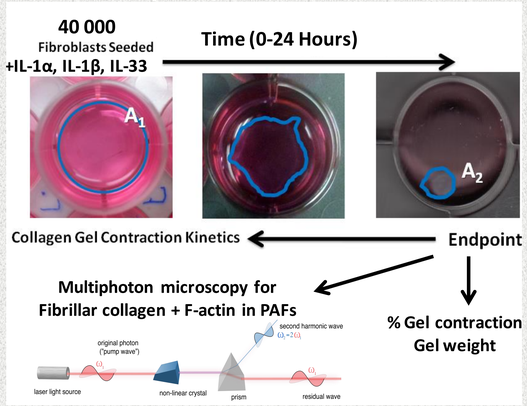


Figure 1. Collagen gel contraction assay. Primary airway fibroblasts (PAFs) are seeded in 0.4mg/ml collagen I gels with or without 1ng/ml IL-1α, IL-1β or IL-33. Gel contraction over 24 hrs was visualized and quantified as the % of the initial gel area using image J software. Contracted collagen I gels were fixed in 4% paraformaldehyde before imaging with second harmonic generation (SHG)- non-linear optical microscopy (NLOM) for fibrillar collagen and confocal imaging with Phalloidin to stain F-actin in lung fibroblasts. Textural analysis was done to assess the Entropy /degree of disorganization of fibrillar collagen after contraction

Increased IL-1α production by the repairing asthmatic airway epithelium

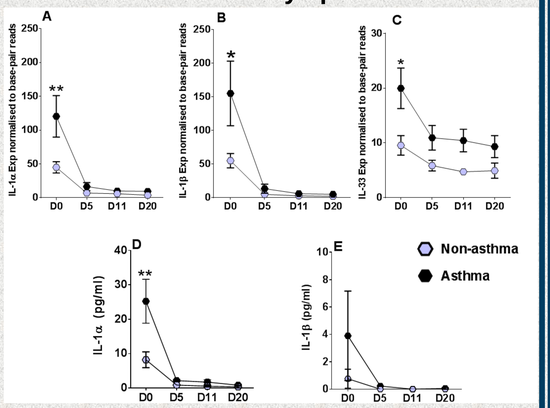


Figure 2. IL-1 & IL-33 production in PAEC ALI cultures. Primary airway epithelial cells (PAECs) from non-asthmatics (n=5) and asthmatics (n=10) were cultured at air-liquid interface. RNA & supernatant collected at Days 0, 5, 11 and 20. **A)** IL-1α, **B)** IL-1β & **C)** IL-33 expression from PAECs expressed as normalized base pair reads. The concentration of **D)** IL-1α & **E)** IL-1β released from PAECs was measured by ELISA. No significant expression or release of other IL-1 family members was detected. Medians ± IQR shown *=P<0.05 & **=P<0.01

IL-1 stimulates inflammatory & growth factor release from airway fibroblasts

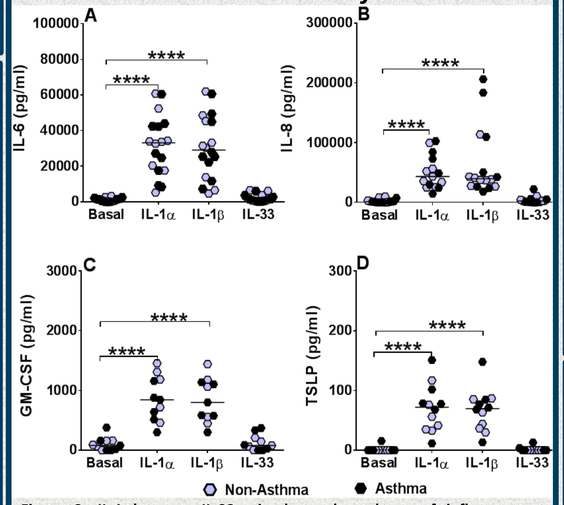


Figure 3. IL-1 but not IL-33 stimulates the release of inflammatory mediators from primary airway fibroblasts (PAFs). PAFs from asthmatics and non-asthmatics were grown to confluence on collagen I coated plates and stimulated with or without 1ng/ml recombinant human IL-1α, IL-1β or IL-33 for 24hours. Concentration of **a)** IL-6, **b)** CXCL8/IL-8, **c)** granulocyte-monocyte colony stimulating factor (GM-CSF) & **d)** thymic stromal lipoprotein (TSLP) released from primary airway fibroblasts after 24 hours. (n=10) ****=P<0.0001

IL-1 induce decreased ECM and GLI-1 expression in airway fibroblasts

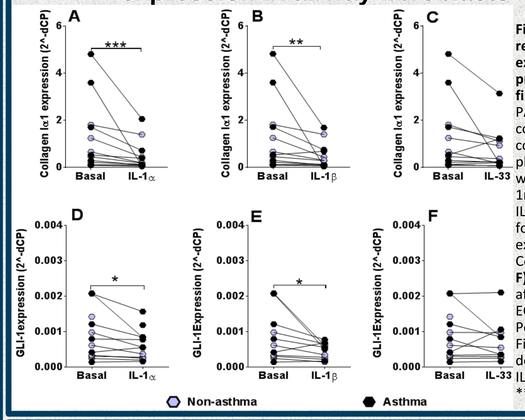


Figure 4. IL-1 down-regulates the expression ECM in primary airway fibroblasts (PAFs) . PAFs were grown to confluence on collagen I coated plates and stimulated with or without 1ng/ml recombinant IL-1α, IL-1β & IL-33 for 24hours. mRNA expression of **(A –C)** Collagen Iα1 and **(D-F)** GLI-1 was assessed after 24 hrs. Other ECM proteins Periostin & Fibronectin were also downregulated upon IL-1 stimulation ***=P<0.001

IL-1 but not IL-33 inhibit airway fibroblast contraction of Collagen I gels

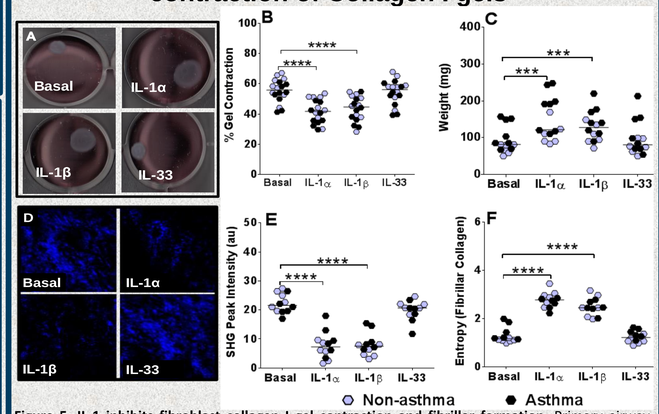


Figure 5. IL-1 inhibits fibroblast collagen I gel contraction and fibrillar formation. Primary airway fibroblasts were seeded in collagen I gels in the presence or absence of 1ng/ml IL-1α, IL-1β or IL-33 and allowed to contract for 24 hours. **A)** Representative gel contraction images, **B)** % gel contraction of gels **C)** semi-dry weight of contracted gels, **D)** Representative images of fibrillar collagen 1 taken with SHG-NLOM, **E)** SHG peak intensity of fibrillar collagen I in contracted gels, **F)** Entropy score for collagen I fibre orientation after textural analysis in contracted gels. (n=10) ****=P<0.0001

IL-1 alters fibroblast morphology in Collagen I gels

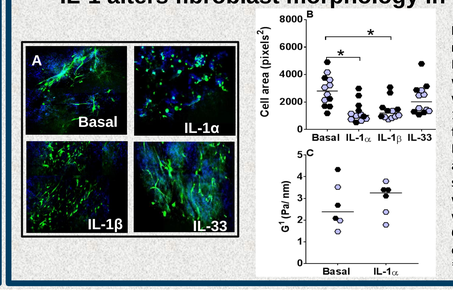


Figure 6. IL-1 alters fibroblast morphology in Collagen I gels. Primary airway fibroblasts (PAFs) were seeded in collagen I gels with or without 1ng/ml IL-1α, IL-1β or IL-33 & allowed to contract for 24 hrs **A)** composite images of PAFs & fibrillar collagen I, **B)** Cell area measured as pixels² of PAFs seeded in collagen I gels. **C)** PAFs were seeded on 96 well plates with or without 1ng/ml IL-1α. Cell stiffness measured with optical magnetic twisting. *=P<0.05

IL-1 controls fibroblast repair phenotype by regulating Lysyl oxidase (LOX) & microtubule formation

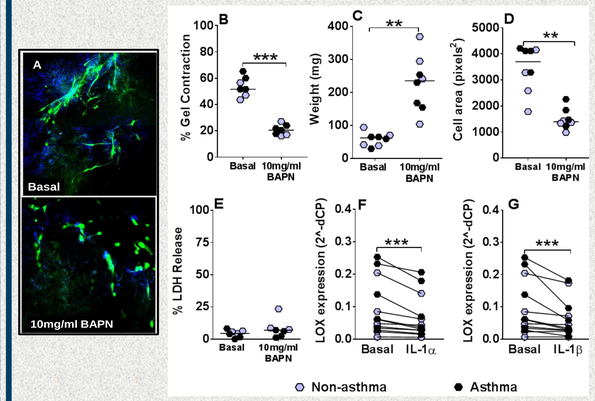
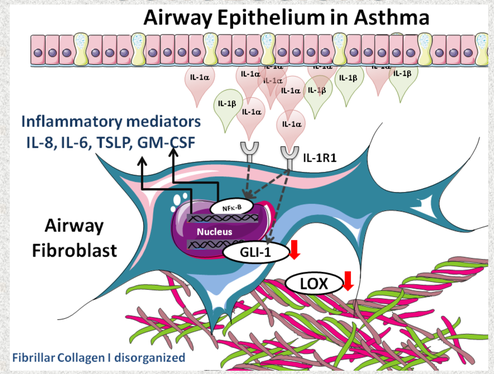


Figure 7. Lysyl oxidase (LOX) is essential for IL-1-dependent regulation of fibroblast remodeling of fibrillar Collagen I. Primary airway fibroblasts (PAFs) were grown to confluence and seeded in collagen I gels and allowed to contract for 24 hrs in the presence or absence of 10mg/ml β-aminopropionitrile (BAPN) which inhibits lysyl oxidase activity **A)** Representative of composite images of fibroblasts and fibrillar collagen 1 taken with SHG-NLOM, **B)** % gel contraction of collagen I gels, **C)** semi-dry weight of contracted gels, **D)** Cell area measured as pixels² of fibroblasts seeded in collagen I gels after contraction. PAFs were grown to confluence on collagen I coated plates. **E)** % lactate dehydrogenase (LDH) released from cells after stimulating with 10mg/ml BAPN for 24 hrs, **F)** mRNA expression of LOX after stimulating PAFs with IL-1α for 24 hours **G)** mRNA expression of LOX after stimulating PAFs with IL-1β for 24 hrs. *=P<0.05, ***=P<0.001

CONCLUSIONS



- **There is an increased production of IL-1α in the repairing asthmatic airway epithelium.**
- **IL-1α and IL-1β but not IL-33 induce inflammatory cytokine release and downregulate ECM production through GLI-1 in airway fibroblasts.**
- **IL-1 inhibits lysyl oxidase expression leading to inhibition of collagen I contraction potentially through inhibition of the fibroblast microtubule cytoskeleton.**
- **This may contribute to increased inflammation as well as defective remodeling of the Collagen I in the asthmatic EMTU.**

REFERENCE: 1. Osei et al., (2016) Interleukin-1α drives the dysfunctional cross-talk of the airway epithelium and lung fibroblasts in COPD. ***Eur Respir J* 48 (1): 350-358**