Interleukin-1 regulation of fibroblast repair phenotype through lysyl oxidase contributes to fibrillar collagen I disorganization in asthmatic airways

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INTRODUCTION

Asthma is a chronic incurable inflammatory disease associated with remodeling of the airways which is not reversible with current therapy. A disturbed epithelial-fibroblast cross-talk in the epithelial-mesenchymal trophic unit (EMTU) is suggested to contribute to airway remodeling in asthma. We recently demonstrated that epithelial-derived-IL-1α is essential for epithelial-fibroblast communication in the EMTU and found an increased production of IL-1α as well as family members IL-1β and IL-33 in asthmatic-derived airway epithelial cells during epithelial repair[1].

AIM

The aim of this study was to assess the mechanism through which IL-1 affects fibroblast repair and remodeling of collagen I and what this means for fibrillar collagen organization in airway remodeling in asthma.

MATERIALS AND METHODS

- Primary airway fibroblasts (PAFs) from non-asthmatic and asthmatic donor lungs seeded in collagen I gels or on collagen I coated 6-well tissue culture plates, were stimulated with media control, 1ng/ml IL-1α, IL-1β, IL-33 or 10mg/ml β-amino propionitrile (BAPN) (a broad inhibitor of lysyl oxidase) for 24 hours.
- Collagen I gel contraction was quantified over 24 hours using Image J and PAFs in gels were stained with Phalloidin for F-actin and imaged with the confocal microscope.
- Formation of fibrillar collagen I fibers was assessed using second harmonic generation non-linear optical microscopy (SHG-NLOM).
- PAFs on collagen I coated plates were assessed for mRNA expression of LOX and supernatant medium was assessed for lactate dehydrogenase (LDH) release from cells.
- Airway sections from the same donor lungs used for PAF isolations were imaged for fibrillar collagen organization in the asthmatics compared to non-asthmatics.

Collagen I gel contraction assay

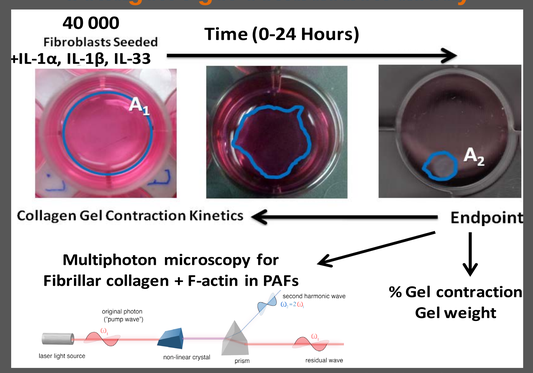


Figure 1. Collagen gel contraction assay. Primary airway fibroblasts (PAFs) are seeded in 0.4mg/ml collagen I gels with or without 1ng/ml IL-1α, IL-1β or IL-33. Gel contraction over 24 hrs was visualized and quantified as the % of the initial gel area using image J software. Contracted collagen I gels 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

IL-1 inhibits airway fibroblast contraction of Collagen I gels

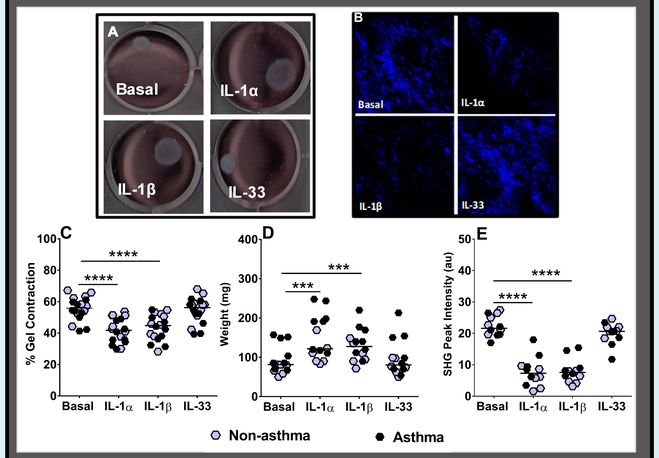


Figure 2. IL-1 inhibits fibroblast collagen I gel contraction and fibrillar formation. Primary airway fibroblasts were seeded in collagen I gels in the presence or absence of 1ng/ml IL-1α, IL-1β or IL-33 and allowed to contract for 24 hours. A) Representative gel contraction images, B) Representative images of fibrillar collagen 1 taken with SHG-NLOM C) % gel contraction of gels D) semi-dry weight of contracted gels E) SHG peak intensity of fibrillar collagen I in contracted gels ****=P<0.0001

IL-1 alters fibroblast morphology and cause fibrillar collagen disorganization in Collagen I gels

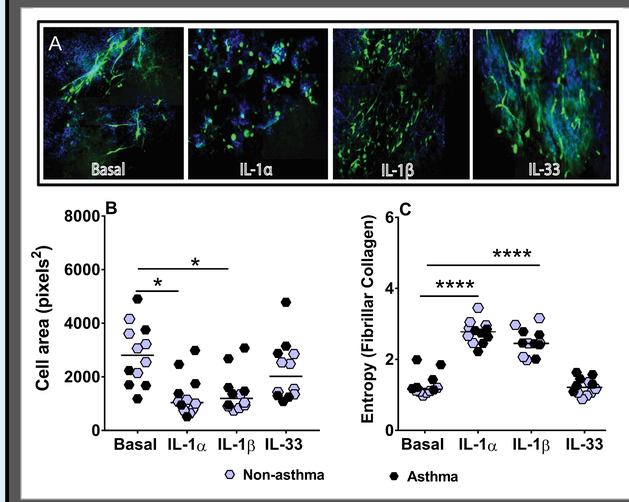


Figure 3. IL-1 alters fibroblast morphology and fibrillar collagen I organization in Collagen I gels. Primary airway fibroblasts (PAFs) were seeded in collagen I gels with or without 1ng/ml IL-1α, IL-1β or IL-33 & allowed to contract for 24 hours A) composite images of PAFs & fibrillar collagen I, B) Cell area measured as pixels² of PAFs seeded in collagen I gels. C) Entropy score for collagen I fiber orientation after textural analysis. *=P<0.05

IL-1 inhibits LOX and GLI-1 expression in airway fibroblasts

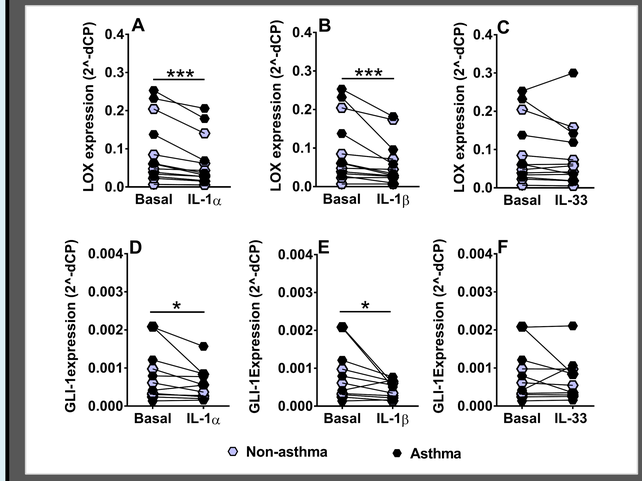


Figure 4. IL-1 suppresses the expression Lysyl oxidase (LOX) and glioma-associated oncogene homolog 1 (GLI-1) expression in primary airway fibroblasts (PAFs). PAFs were grown to confluence on collagen I coated plates and stimulated with or without 1ng/ml recombinant IL-1α, IL-1β & IL-33 for 24hours. mRNA expression of (A–C) Lysyl oxidase and (D-F) GLI-1 was assessed after 24 hrs. ***=P<0.001

LOX inhibition suppresses fibroblast collagen I contraction

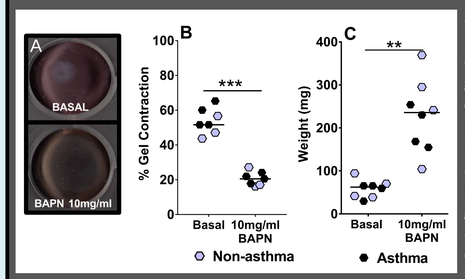


Figure 5. LOX inhibitor β-aminopropionitrile (BAPN) inhibits PAF contraction of collagen I gels. PAFs were seeded in collagen I gels in the presence or absence of 10mg/ml BAPN and allowed to contract for 24 hours. A) Representative gels B) % gel contraction & C) Semi-dry weight of contracted gels ****=P<0.0001

LOX inhibition causes abnormal fibroblast repair and disorganization of fibrillar collagen I

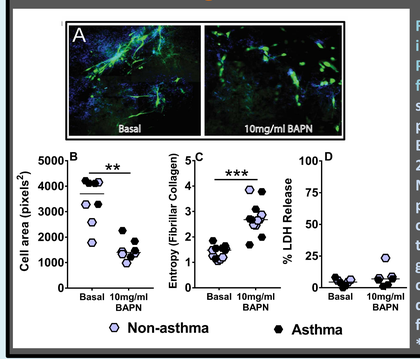


Figure 6. Lysyl oxidase (LOX) inhibitor BAPN causes abnormal PAF morphology and repair of fibrillar collagen I. PAFs were seeded in collagen I gels in the presence or absence of 10mg/ml BAPN and allowed to contract for 24 hours. A) Representative SHG-NLOM images. B) Cell area in pixels² of PAFs C) Entropy score for collagen I fiber orientation after textural analysis D) PAFs were grown to confluence on collagen I coated plates. Percentage lactate dehydrogenase (LDH) released from cells after 24 hours. ****=P<0.0001

Fibrillar collagen I is increased & disorganized in asthmatic airways

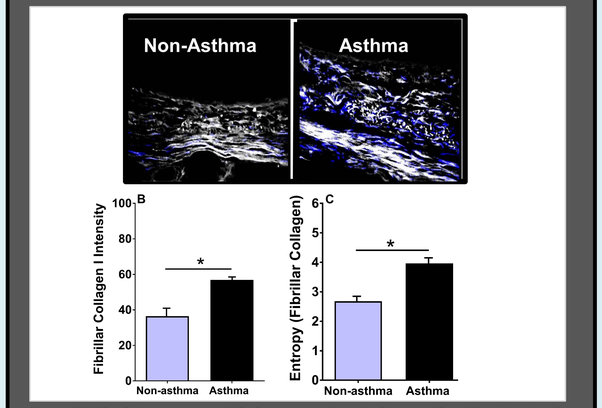
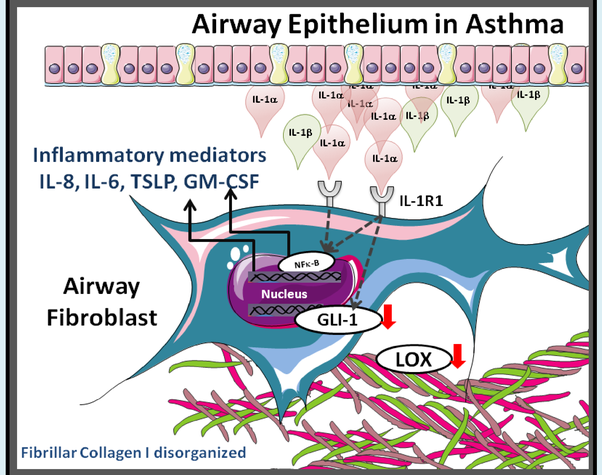


Figure 7. Fibrillar collagen I is increased and disorganized in the lamina propria of asthmatics. A) Representative SHG-NLOM images of the lamina propria of the large airways comparing non-asthmatics to asthmatics B) Mean levels of fibrillar collagen intensity in blue and C) Entropy score for collagen I fiber orientation after textural analysis. *=P<0.05

CONCLUSIONS



- IL-1α and IL-1β but not IL-33 inhibits airway fibroblast repair and contraction of fibrillar collagen I.
- IL-1 inhibits lysyl oxidase and GLI-1 expression leading to inhibition of collagen I contraction potentially through inhibition of the fibroblast morphology microtubule cytoskeleton.
- IL-1 induced abnormal fibroblast repair of fibrillar collagen I through lysyl oxidase may explain fibrillar collagen I disorganization in the airways of asthmatics.
- Disorganized fibrillar collagen I may stimulate an increased deposition of excess fibrillar collagen by airway fibroblasts leading to airway remodeling in asthma.

REFERENCE:

1. Osei et al, (2017). The role of Interleukin-1 in driving inflammation and remodeling in the asthmatic EMTU. American Journal of Respiratory Critical Care Medicine 2017:195 A7248