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.

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 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 oxydase) for 24 hours.

• Collagen I gel contraction was quantified over 24 hours using time-lapse imaging with Phalloidin to stain F-actin in lung fibroblasts. Textural analysis was done to assess collagen I fiber orientation after textural analysis.

• 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 supermatant 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.

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

Collagen I gel contraction assay

40 000 Fibroblasts seeded IL-1α, IL-1β, IL-33

Time (0-24 Hours)

Collagen Gel Contraction Kinetics

% Gel contraction

Gel weight

% Gel contraction vs Gel weight

Collagen Gel Contraction Kinetics

Multimophon microscopy for Fibrillar collagen I-F-actin in PAFs

% Gel contraction

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 I taken with SHG-NLOM C) % gel contraction of 2D semi-dry weight of contracted gels D) SHG peak intensity of fibrillar collagen I in contracted gels. *** P<0.001

IL-1 inhibits airway fibroblast contraction of Collagen I gels

Asthma

Non-asthma

Figure 3. IL-3 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 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

CONCLUSIONS

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

IL-1 regulates fibrilloblast repair phenotype through lysyl oxydase driven collagen I disorganization in asthmatic airways

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INflammatory mediators

Airway Epithelium in Asthma

Airway Fibroblast

Fibrillar Collagen I disorganized

Airway Fibroblast

Collagen I gel disorganization

Lysyl oxidase (LOX) inhibitor 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 gel contraction images, B) Mean levels of fibrillar collagen intensity in blue and C) Entropy score for collagen I fiber orientation after textural analysis. *** P<0.001

LOX inhibition suppresses fibroblast collagen I contraction

Figure 5. LOX inhibitor β-amino propionitrile (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 gel contraction images, B) Mean levels of fibrillar collagen intensity in blue and C) Entropy score for collagen I fiber orientation after textural analysis. *** P<0.001

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 in SHG-NLOM images. A) Cell area measured as pixels2 of PAFs in collagen I gels, B) Entropy score for collagen I fiber orientation after textural analysis. *** P<0.001

Airway Fibroblast

Collagen I gel disorganization

IL-1α and IL-1β but not IL-33 inhibits airway fibroblast repair and contraction of fibrillar collagen I

IL-1α and IL-1β inhibits lysyl oxidase and GLI-1 expression leading to inhibition of collagen I contraction potentially through inhibition of the fibrilloblast morphology microtubule cytoskeleton. IL-1α and IL-1β inhibited abnormal fibroblast repair of fibrillar collagen I through lysyl oxidase may explain fibrilloblast collagen I disorganization in the airways of asthmatics.

Disorganized fibrilloblast collagen I may stimulate an increased deposition and disorganization fibrillar collagen by airway fibroblasts leading to airway remodeling in asthma.

REFERENCES