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Segmental allergen challenge increases levels of airway FSTL1 in asthmatics

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CAPSULE SUMMARY

Segmental allergen challenge induced increased levels of bronchoalveolar (BAL) follistatin like 1 (FSTL1). BAL macrophages both expressed FSTL1, and could be induced by FSTL1 to express MMP9 suggesting a potential role of FSTL1 in remodeling.

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Keywords

Follistatin like 1; Macrophage; MMP9

To the Editor

We have used a proteomic approach to identify novel mediators of inflammation in the sputum of an asthma compared to a healthy control subject (Supplementary Methods E1, E2). Using this approach we determined that follistatin like 1 (FSTL1) is >200 fold more highly expressed in the asthma compared to the control subject and was the most highly expressed of the 508 proteins we examined in sputum. FSTL1, a 308 amino acid extracellular glycoprotein that shares 94% identity in man and mouse[1,2], is generated by non-hematopoietic cells such as cells of the mesenchymal lineage (fibroblasts, chondrocytes, osteocytes, adipocytes, cardiomyocytes) by stimuli including TGFβ, IL1β, TNFα, and IL6[1,2]. FSTL1 released from these mesenchymal cells targets immune cells (monocytes, macrophages and T cells) to express pro-inflammatory cytokines (IL1β, TNFα, IL6, IFNγ) and chemokines (IL8, MCP1, IP10)[1]. FSTL1 has been studied in embryogenesis[E1], tumor development[E2], cardiac disease[E3], arthritis[E4,E5], bleomycin induced lung fibrosis[E6], and wound healing[E7]. The predominant effect of FSTL1 appears to be pro-inflammatory[E4,E5,E8], although anti-inflammatory effects of FSTL1 have also been described[E9]. Using a mouse model we recently demonstrated that allergen challenge induced mouse lung macrophages to highly express FSTL1 which was associated with airway remodeling[3]. Therefore, this study examined whether in humans, like in mice, allergen challenge could also induce expression of FSTL1 in the airway, and whether human bronchoalveolar lavage (BAL) macrophages like mouse macrophages expressed FSTL1 and were also activated by FSTL1 to express pro-remodeling mediators such as MMP9[4].

To determine whether allergen challenge induced lung expression of FSTL1, stored BAL fluid samples from 12 mild allergic asthma subjects (Table E1) were assayed for FSTL1 by ELISA (R&D) in a protocol approved by the University of Wisconsin-Madison Health Sciences Institutional Review Board. In brief, segmental bronchoprovocation with allergen (SBP-Ag) and BAL (pre- and post-allergen) was performed (Supplementary Methods E3, E4) as previously described[5]. SBP-Ag in asthmatics induced a significant increase in levels of BAL FSTL1 protein ($p<0.03$) (pre-allergen vs post-allergen) (n=12 subjects) (Figure 1A). Nine additional asthma subjects previously described [E10] who had sufficient BAL cells were included to analyze FSTL1 mRNA expression before and after SBP-Ag. There was an increase in FSTL1 mRNA in BAL cells after SBP-Ag (assessed by RT-qPCR) (Supplementary Methods E4, E5, E6) which approximated statistical significance ($p=0.057$) (pre-allergen vs post-allergen) (n=9 subjects) (Figure 1B), and was positively associated with the percentage of BAL macrophages (Table E2). Levels of BAL cell FSTL1 mRNA correlated significantly with levels of BAL cell MMP9 mRNA ($r=0.67$; $p= 0.04$) (Figure 1F), but not with BAL MMP9 protein (not shown). The negative correlation between FSTL1 and eosinophils (Table E2) could indicate that FSTL1 may also have anti-inflammatory effects on eosinophils. In this regard, at least two studies of FSTL1 administration in mouse models of arthritis have demonstrated that FSTL1 has anti-inflammatory effects as

demonstrated by reduced joint inflammation and reduced expression of IL-6 and PGE2[E11, E12].

To determine whether human lung BAL macrophages expressed FSTL1 we utilized two approaches, immunostaining of post-mortem human lungs of asthmatics to detect whether FSTL1 was expressed by alveolar macrophages, and investigation of whether post-mortem human BAL macrophages expressed FSTL1. We immunostained post-mortem lung sections (asthma and normal control) (n=3/group) obtained from National Disease Research Interchange (Philadelphia, PA) with an anti-FSTL1 Ab (R&D) (Supplementary Methods E7) (Figure 1C–E). These studies demonstrated that FSTL1 was highly expressed in lung alveolar macrophages in asthma (Figure 1E), but was not significantly expressed in control lung alveolar macrophages (Figure 1C). The number of FSTL1+ cells were significantly higher in the airway of lungs of asthma compared to controls ($p<0.001$) (n=3) (Figure 1E)

To determine whether human BAL macrophages responded to FSTL1, BAL macrophages were obtained from human lungs by lavage post-mortem (Supplementary Methods E8) as previously described[6]. Incubation of BAL macrophages with FSTL1 (100 ng/ml) induced a significant increase in levels of macrophage FSTL1 mRNA as assessed by RT-qPCR as compared to macrophages cultured in media alone ($p<0.05$) (Figure 2A). TGFβ1 a known inducer of FSTL1, also significantly induced FSTL1 mRNA expression by BAL macrophages ($p<0.05$) (Figure 2A). These studies suggest that macrophage derived FSTL1 can either through paracrine or autocrine mechanisms induce further FSTL1 expression by macrophages, similar to what we have noted in studies of mouse macrophages[3]. In addition, FSTL1 induced BAL macrophages to express MMP9 mRNA as assessed by RT-qPCR ($p<0.05$) (Figure 2B), as well as MMP9 protein as quantitated by ELISA (R&D) ($p<0.05$) (Figure 2C). Previous studies have demonstrated that FSTL1 can activate Toll like receptor 4 (TLR4) dependent cytokine responses in cell types other than human BAL macrophages[E13]. Accordingly we incubated BAL macrophages (n=6 donors) with FSTL1 and an anti-TLR4 Ab and noted significantly reduced levels of FSTL1 mRNA ($p<0.02$) (Figure 2D) and MMP9 mRNA ($p<0.05$) (Figure 2E). Thus, blocking only TLR4 signaling inhibits FSTL1 activation of BAL macrophages in vitro. Further studies are needed to determine whether other TLR4 expressing cells in the lung that respond to FSTL1 do so through TLR4 and/or other known FSTL1 signaling pathways (i.e. bone morphogenic protein, protein kinase B, adenosine monophosphate activated protein kinase, Na/K-ATPase membrane potential)[E13–E16].

In summary, we have made the novel observation that segmental allergen challenge increases levels of FSTL1 protein in the airway of asthmatics, and that levels of human BAL cell FSTL1 mRNA correlate with levels of BAL cell MMP9 mRNA, a remodeling mediator we demonstrate is induced in macrophages by FSTL1 in vitro. In addition, we show that human lung airway macrophages express and respond to FSTL1, potentially through paracrine or autocrine pathways. This study also demonstrates that human lung alveolar macrophages can be induced to express FSTL1 which suggests a different cellular source of FSTL1 in asthma compared to other diseases (arthritis, auto-immune disease, coronary disease) where macrophages and myeloid cells are not a significant source of FSTL1[1,2]. FSTL1 in these diseases is generated in particular by non-hematopoietic cells such as cells

of the mesenchymal lineage (fibroblasts, chondrocytes, osteocytes, adipocytes, cardiomyocytes)[1]. The potential functional significance of allergen challenge inducing FSTL1 is suggested from our studies demonstrating that in humans FSTL1 can induce BAL macrophages to express MMP9 a metalloproteinase associated with remodeling in asthma[7–9]. The present human study demonstrates that allergen challenge of subjects with asthma induces FSTL1 expression, and extend our observations in mice where we have demonstrated that mouse Fstl1 promotes airway remodeling[3]. Further study is needed to determine whether targeting FSTL1 would inhibit airway remodeling in humans with asthma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

$AgPD_{20}$	Provocative dose of allergen leading to a 20% fall in FEV_1
BAL	Bronchoalveolar lavage
FSTL1	Follistatin like 1
MMP9	Matrix metalloproteinase 9
SBP-Ag	Segmental bronchoprovocation with allergen

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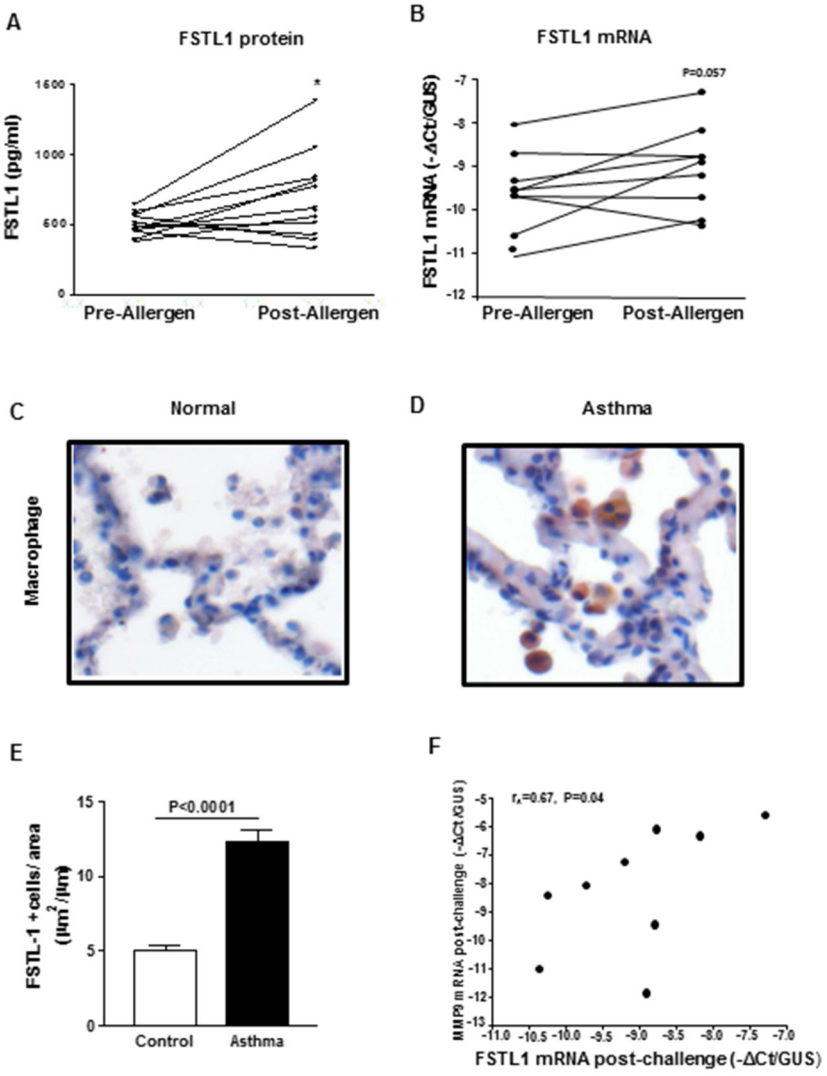

Figure 1. Segmental allergen challenge increases levels of FSTL1

A. Levels of FSTL1 were quantitated by ELISA in BAL fluid obtained before and after segmental allergen challenge in allergic asthmatic subjects ($p<0.03$) (n=12 subjects). **B.** Levels of FSTL1 mRNA were quantitated by RT-PCR in BAL cells obtained before and after segmental allergen challenge in allergic asthmatic subjects ($p=0.057$) (n=9 subjects). **C–E.** Lung sections from a normal subject and an asthmatic were immunostained with an anti-FSTL1 antibody and demonstrate FSTL1+ positive alveolar macrophages in the asthmatic **(D),** but not in the normal subject (**C**). The number of FSTL1+ alveolar macrophages quantitated by image analysis (**E)** were significantly higher in the lungs of asthma compared to controls ($p<0.001$) (n=3 subjects/group). **F.** BAL cell FSTL1 and MMP9 mRNA levels after segmental allergen challenge were determined by RT-PCR, and correlation calculated using Spearman's correlation ($r=0.67$; $p=0.04$) (n=9 subjects).

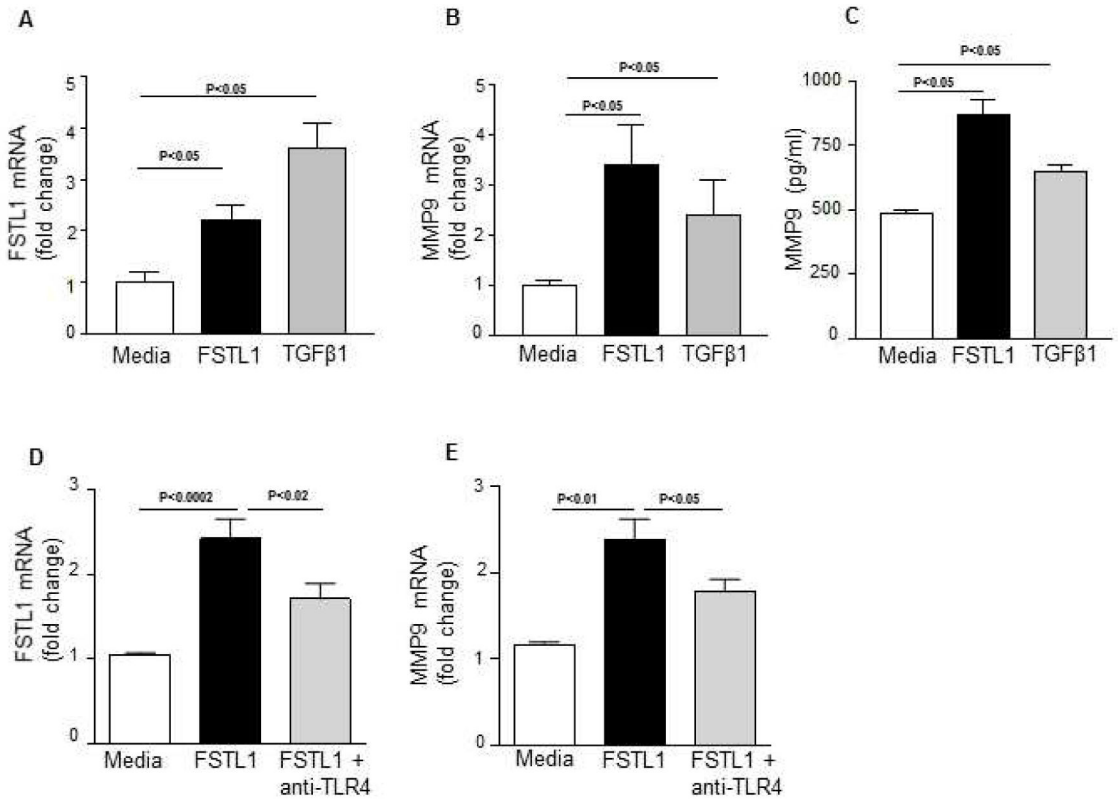

Figure 2. FSTL1 induces expression of FSTL1 and MMP9 by BAL macrophages in vitro

A–C. Human BAL macrophages (n=3 subjects) were incubated in vitro with either FSTL1 (100 ng/ml) or TGFβ1 (100 ng/ml). Levels of FSTL1 mRNA (**A)** and MMP9 mRNA **(B)** were quantitated by RT-qPCR, and levels of MMP9 protein (**C)** by ELISA. (**D–E).** Human BAL macrophages (n=6 subjects) were incubated in vitro with either an anti-hTLR4-IgG neutralizing Ab, or a species and isotype control mouse IgG Ab, for 1 hour prior to stimulation with FSTL1 (100 ng/ml) for 24 hours. Levels of FSTL1 mRNA (**D)** and MMP9 mRNA **(E)** were quantitated by RT-qPCR.