Airway factor XIII associates with type 2 inflammation and airway obstruction in asthmatic patients

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Background: Coagulation Factor XIII (FXIII) plays an important role in wound healing by stabilizing fibrin clots and cross-linking extracellular matrix proteins. FXIII is expressed in cells of the monocyte/macrophage and dendritic cell lineages in response to type 2 cytokines.
Objective: We sought to determine the association between FXIII and asthma pathobiology.
Methods: We analyzed the expression of FXIII mRNA and protein levels in bronchoalveolar lavage samples obtained before and after segmental allergen challenge from patients with mild asthma and in induced sputum samples collected from patients with mild-to-moderate and severe asthma.
Results: FXIII mRNA and protein levels were highly upregulated in bronchoalveolar cells and fluid after allergen challenge and mRNA levels correlated with protein levels. In sputum of asthmatic patients, FXIII expression was positively correlated with type 2 immune response and dendritic cell markers (CD209 and CD207). FXIII expression was also associated with increased airflow limitation (FEV_1/forced vital capacity and residual volume/total lung capacity ratios) and greater reversibility to β-agonists.
Conclusions: FXIII expression was upregulated in the airways of asthmatic patients after allergen exposure. Expression in the sputum of asthmatic patients correlated with the type 2 immune response and airflow limitation. Excessive activity of FXIII could contribute to the pathophysiology of airway obstruction in asthmatic patients. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *Factor XIII, asthma, severe asthma, allergy, pulmonary function, airway obstruction, air trapping, inflammation, eosinophils, IL-13*

Abbreviations used
BAL: Bronchoalveolar lavage
Ct: Cycle threshold
DC: Dendritic cell
DC-SIGN: Dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegrin
FXIII: Coagulation Factor XIII
FVC: Forced vital capacity
qPCR: Quantitative PCR
RT: Reverse transcription
RV: Residual volume
SARP: Severe Asthma Research Program
SBP-Ag: Segmental bronchoprovocation with an allergen
TLC: Total lung capacity

Asthma is characterized by both airway inflammation and remodeling. Airway remodeling is reflected by thickening of the reticular basement membrane, mucus gland hypertrophy, deposition of extracellular matrix, increased smooth muscle mass, and angiogenesis.[1-4] Collectively, these changes contribute to persistent airflow limitation and asthma severity.

Airway inflammation is associated with plasma extravasation, and exposure of plasma to tissue factor triggers a cascade of coagulation factors that leads to thrombin activation and fibrin clotting.[5] We and others have reported increased thrombin activity in airways of asthmatic patients.[6,7] Wagers et al[8] have observed fibrin deposition in the airway of a patient who died in status asthmaticus. Mechanistically, the study by Wagers et al suggested that fibrin reduces surfactant function, which then ultimately leads to airway closure and hyperresponsiveness. In patients with severe asthma, the profibrinogenic pathway is increased compared with that in patients with less severe disease.[9,10]

Coagulation Factor XIII (FXIII) covalently cross-links fibrin at the end of the coagulation cascade. FXIII is a transglutaminase present extracellularly as plasma FXIII and intracellularly as cellular FXIII. Plasma FXIII is activated by thrombin and possesses a multitude of substrates that participate in the stability of the fibrin clot during the wound-healing process.[11,12] These substrates include fibronectin, thrombospondin 1, α_2-antiplasmin, thrombin-activatable fibrinolysis inhibitor, actin, von Willebrand factor, and plasminogen activator inhibitor 2. Because of its function to stabilize fibrin clots, FXIII plays a major role in acute thrombotic events, such as myocardial infarction, ischemic stroke, deep vein thrombosis, and pulmonary embolism.[13] Furthermore, fibrin plugs with and without eosinophilic inflammation are found in patients with life-threatening plastic bronchitis, which is seen in those with several pulmonary diseases, including asthma.[14,15] However, the presence and role of FXIII in the airways of asthmatic patients have yet to be analyzed. *In vitro* allergen-activated PBMCs

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Supported by Severe Asthma Research Program Grants R01 HL069116 and U10 HL109168, Program Project Grant P01 HL088594, and Clinical and Translational Research Center Grants UL1 RR025011 and UL1 TR000427 from the National Institutes of Health.

Disclosure of potential conflict of interest: W. W. Busse has received consultancy fees from Novartis, GlaxoSmithKline, Genentech, Roche, Pfizer, Merck, Boehringer-Ingelheim, Sanofi, AstraZeneca, Gilead, Teva Pharmaceuticals, Takeda, and Aerocrine; and is a member of Data Monitoring Boards and Study Oversight Committee for Boston Scientific, Circassia, and ICON. N. N. Jarjour has received consultancy fees from Asthmatx, Genentech, and Teva Pharmaceuticals. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication January 26, 2015; revised April 30, 2015; accepted for publication May 1, 2015.

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0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2015.05.053

from asthmatic patients express more FXIII mRNA than untreated cells.[16] Also, FXIII protein levels were augmented in bronchoalveolar lavage (BAL) fluid from children with bronchoalveolar inflammation compared with those in lavage fluid from healthy children.[17] Recently, expression quantitative trait locus mappings identified cis-acting expression-associated variants in FXIII in relationship to asthma pathogenesis in children.[18] In addition, recently, accumulation of $FXIII^+$ dendritic cells was reported in lung tissue from patients who died from asthma.[19]

FXIII expression is upregulated in IL-4– and IL-13–activated macrophages,[20,21] suggesting FXIII is a marker of alternatively activated macrophages. Production of FXIII in alternatively activated macrophages was further confirmed in nasal polyps from patients with chronic rhinosinusitis,[22] in which FXIII is thought to contribute to fibrin deposition. Using whole human genome expression microarrays, we have previously shown that FXIII was one of the transcripts upregulated in BAL cells after *in vivo* segmental bronchoprovocation with an allergen (SBP-Ag) in asthmatic patients.[23]

On the basis on these observations, we hypothesized that FXIII production by airway cells might be associated with type 2 immune characteristics and a loss of pulmonary function in asthmatic patients. To test this hypothesis, we analyzed FXIII expression in both BAL fluid after SBP-Ag and induced sputum samples from a group of asthmatic patients enrolled in the Severe Asthma Research Program (SARP) at the University of Wisconsin.

METHODS

Subjects, BAL cell preparations, and study designs

The study protocol was approved by the University of Wisconsin–Madison Health Sciences Institutional Review Board. Informed written consent was obtained from subjects before participation.

All 7 subjects undergoing SBP-Ag and BAL were atopic, with at least 1 positive skin prick test response. These subjects had a history of mild asthma with airway reversibility to albuterol. None of the subjects were using inhaled or oral corticosteroids. Detailed methods for bronchoscopy, SBP-Ag, and BAL cell preparation have previously been described.[24] Blood eosinophils were purified by means of negative selection, as previously described.[25] More details are provided in the Methods section in this article's Online Repository at www.jacionline.org.

Induced sputum was obtained by using standard methods[26,27] from 56 subjects enrolled in SARP at the University of Wisconsin. Additional details are provided in the Methods section in this article's Online Repository. These subjects had severe (n = 22) or nonsevere asthma (n = 34), as defined by the American Thoracic Society criteria. Sputum samples were processed in 2 sets. Set 1 (n = 23) includes sputum samples obtained over a period of 12 months (2010-2011). Set 1 was used for association analyses between transcripts. Thirty-three sputum samples (set 2) obtained between 2007 and 2010 were added to samples from set 1 for association analyses between FXIII with asthma characteristics. The subjects' characteristics were obtained as described in the Methods section in this article's Online Repository.

RNA, reverse transcription quantitative PCR, and ELISA

Total RNA was extracted from unfractionated BAL cells or purified BAL eosinophils by using the RNeasy Mini Kit (Qiagen, Valencia, Calif). Total RNA from sputum samples was extracted according to the recommendations of the TRIzol Reagent manufacturer (Invitrogen, Carlsbad, Calif). Typically, 400 to 800 ng of total RNA was recovered from the sputum samples. The reverse transcription (RT) reaction was performed by using the Superscript III system (Invitrogen/Life Technologies, Grand Island, NY). Expression of mRNA was determined by using quantitative PCR (qPCR) with SYBR Green Master Mix (SABiosciences, Frederick, Md). Data are expressed as fold change by using the comparative cycle threshold ($\Delta\Delta$Ct) method, as described previously.[28] The values presented and used for correlations are fold change of $2^{\Delta\Delta Ct}$ compared with the lowest expression among the asthmatic patients, which was fixed at 1. Sputum samples with housekeeping gene (β-glucuronidase *[GUSB]*) Ct values of greater than 25 were excluded from the study. More details are provided in Table E1 and the Methods section in this article's Online Repository at www.jacionline.org.

FXIII protein levels were measured in BAL fluid by using the Zymutest Factor XIII-A kit (Hyphen Biomed, Neuville-sur-Oise, France). Free FXIII or FXIII complexed with FXIIIB were both measurable with the ELISA kit. Samples were processed, as recommended by the manufacturer. The assay sensitivity was approximately 1 ng/mL.

Statistical analysis

Data were analyzed with the Wilcoxon signed-rank test to compare expression of genes in total BAL cells and purified BAL eosinophils by using RT-qPCR or ELISA. Demographic factors were compared between patients with nonsevere and those with severe asthma by using the Wilcoxon rank sum test and the χ^2 test for association. In sputum FXIII expression levels were compared between groups by using the Wilcoxon rank sum test and compared with continuous measurements by using the Spearman rank correlation coefficient. A trend test for association between increasing inhaled corticosteroid dose and FXIII expression was obtained by regressing log FXIII expression on inhaled corticosteroid dose, where dose levels of none through high were recoded as 0 through 3. Because FXIII expression tended to increase with age, all analyses were performed by using age-adjusted FXIII expression levels, which were calculated by regressing log FXIII expression on age and obtaining standardized residuals. A 2-sided *P* value of less than .05 was regarded as statistically significant.

RESULTS

FXIII expression is increased in BAL fluid after SBP-Ag

FXIII mRNA levels were assessed by using RT-qPCR of unfractionated BAL cells before and 48 hours after SBP-Ag and BAL eosinophils purified 48 hours after SBP-Ag (Fig 1). FXIII mRNA levels increased 300-fold in BAL cells after SBP-Ag compared with those in BAL cells obtained from the same subjects at baseline (before allergen challenge). The main cell population (74% of the total) recruited into the airway 48 hours after SBP-Ag was eosinophils (see Table E2 in this article's Online Repository at www.jacionline.org). FXIII mRNA levels were low in purified BAL eosinophils compared with those in unfractionated BAL cells (Fig 1), demonstrating that eosinophils are not the main source of FXIII mRNA. FXIII protein levels in BAL fluid were close to the sensitivity of the ELISA before SBP-Ag and increased to approximately 30 ng/mL after allergen challenge (Fig 1). In BAL fluid obtained 48 hours after SPB-Ag, FXIII protein levels were correlated to mRNA levels (see Fig E1 in this article's Online Repository at www.jacionline.org). The ratio of albumin to total protein levels was similar in BAL fluid and plasma (see Fig E1). In contrast, the ratio of FXIII to albumin in BAL fluid diverged from the ratio in plasma, suggesting FXIII-expressing cells rather than blood provided FXIII protein in the BAL fluid.

FXIII mRNA levels correlate with expression of markers of the type 2 immune response and markers of the dendritic cell family

In the first set of sputum samples (set 1), 23 asthmatic patients were analyzed for FXIII expression *vis-à-vis* expression of several