CHEST

Airway Surfactant Protein D Deficiency in Adults With Severe Asthma

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BACKGROUND: Surfactant protein D (SP-D) is an essential component of the innate immune defense against pathogens within the airways. SP-D also regulates allergic inflammation and promotes the removal of apoptotic cells. SP-D dysregulation is evident in several pulmonary diseases. Our aim was to investigate whether airway and serum levels of SP-D are altered in treatment-resistant severe asthma.

METHODS: SP-D concentrations were measured in matched serum and BAL samples collected from 10 healthy control subjects (HC) and 50 patients with asthma (22 with mild asthma [MA] and 28 with severe asthma [SA]). These samples were also evaluated by using Western blot analysis to investigate variations in SP-D size.

RESULTS: SP-D levels in BAL samples were significantly lower in SA compared with HC and MA ($P < .001$) and inversely correlated with BAL eosinophil cationic protein concentrations in SA ($P < .01$). Serum SP-D was significantly increased in SA compared with HC and MA ($P < .001$), and BAL/serum ratios were significantly lower in SA compared with HC and MA ($P < .001$). Reduced SP-D levels in BAL samples, with concomitant increases in serum in SA, were associated with degraded fragments of SP-D in the serum and increased BAL neutrophil counts and lipopolysaccharide levels.

CONCLUSIONS: These findings suggest defective innate immunity within the airways in SA, as reflected by low BAL SP-D concentrations and altered bacterial presence with airway neutrophilia. Furthermore, BAL SP-D leakage into the serum in patients with SA may provide a peripheral blood biomarker, reflecting increased epithelial damage and/or epithelial permeability within the peripheral airways. CHEST 2016; 149(5):1165-1172

KEY WORDS: asthma; biomarkers; bronchoalveolar lavage; eosinophilic inflammation; immunology asthma; immunology (lung); neutrophilic inflammation; severe asthma; surfactant protein D

FOR EDITORIAL COMMENT SEE PAGE 1121

ABBREVIATIONS: α-rfhSP-D = antirecombinant fragment human surfactant protein D; ECP = eosinophil cationic protein; IPF = idiopathic pulmonary fibrosis; IQR = interquartile range; LPS = lipopolysaccharide; SP-D = surfactant protein D

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FUNDING/SUPPORT: This work was funded by the following grants: Wessex Severe Asthma Cohort Medical Research Council grant [code G0800649] and A Life Course Approach to Investigating Asthma Pathogenesis and Progression Medical Research Council grant [code G0900453].

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DOI: http://dx.doi.org/10.1016/j.chest.2015.11.012

Asthma is a chronic airway disorder that is characterized pathologically by airway inflammation and structural tissue remodeling. Airway inflammation in asthma is typically eosinophilic, with elevated levels of type 2 cytokines such as IL-4, IL-5, and IL-13.[1] Airway remodeling is characterized by structural airway changes associated with airway wall thickening, which is most evident in patients with severe asthma, and inversely correlates with lung function measures.[2] In severe asthma, there may be neutrophilic as well as eosinophilic airway inflammation.[3] We reported previously that neutrophilic asthma is linked to an altered bacterial profile within recovered BAL samples.[4] Because surfactant protein D (SP-D) is an important component of innate immunity within the distal airways, we investigated the potential that dysregulation of airway SP-D is a feature of severe asthma.

SP-D has numerous actions whose deficiency could be detrimental in asthma. In the airways, SP-D binds to carbohydrates in a calcium-dependent manner, orchestrating pathogen aggregation and enhancing their phagocytosis.[5] In addition, SP-D enhances their chemotactic, phagocytic, and oxidative properties and interacts with phagocytic cells, such as macrophages and neutrophils.[6] SP-D also modifies allergic responses in that it reduces the activation of basophils,[7] mast cells,[8] and eosinophils[9] while regulating the release of transforming growth factor-β,[10] IL-10,[11] and IL-12[12] and reducing concentrations of IL-2.[13] In allergen challenge models, exogenous SP-D inhibits allergen-induced T lymphocyte proliferation and hypersensitivity,[12] inducing a shift from a type 2 to type 1 cytokine response, and decreases induced airway remodeling.[9,14] Conversely, SP-D-deficient mice exhibit an enhanced IL-13-dependent inflammatory allergic response within the airways.[15]

The aim of the present study was to investigate airway luminal and serum SP-D concentrations in patients with mild and severe asthma compared with healthy control subjects and to examine the structural integrity of SP-D in these study groups. We hypothesized that severe asthma may be associated with a deficiency in SP-D within the airways.

Subjects and Methods

Subjects

Volunteers aged 18 to 65 years (recruited from the Wessex Severe Asthma Cohort and a departmental database of volunteers) participated in the study, which had previously received ethics approval from Southampton and West Hampshire Research Ethics Committee A (reference numbers 05/Q1702/165 and 08/H0502/6). All subjects provided written informed consent. The healthy control subjects had no history of respiratory disease and no evidence of bronchial hyperreactivity to methacholine challenge. Volunteers with asthma all had a physician-confirmed diagnosis of the disease. In addition, subjects with mild asthma all tested positive on skin prick tests to house dust mite allergen extract (*Dermatophagoides pteronyssinus*), had abnormal airway reactivity to methacholine required to lower the FEV_1 by 20% of < 8 mg/mL, were life-long nonsmokers, and only receiving (as required) short-acting β-agonist therapy. Patients with severe asthma were on step 4 ($n = 12$) or step 5 ($n = 16$) of the Global Initiative for Asthma guidelines therapy[16]; were poorly controlled, with a score ≥ 1.5 on the six-domain Asthma Control Questionnaire; and had not smoked for at least 1 year.

Bronchoscopic Airway Samples

Fiberoptic bronchoscopy was performed according to established guidelines.[17] BAL was performed by instilling 6 × 20-mL aliquots of prewarmed normal saline into a subsegmental bronchus of the anterior segment of the right upper lobe followed by gentle suction. BAL fluid was filtered (BD Falcon Cell Strainer) and then centrifuged at 800*g* for 10 min at 4°C. Cell pellets were resuspended in phosphate-buffered saline for cytospins, and the supernatant was stored at −80°C for later analysis. Cells were stained by using a rapid Romanowsky stain (Raymond Lamb Ltd) to distinguish between macrophages, neutrophils, and eosinophils, and 400 cells were counted blind by using coded samples.

Serum Sampling

Venous blood was allowed to clot for 60 min and then centrifuged for 15 min at 1,500*g* at 4°C. The serum layer was removed and stored at −80°C for further analysis.

SP-D Enzyme-Linked Immunosorbent Assay

Antibodies were raised in rabbits against a recombinant fragment of SP-D (neck/head), which is considered the functional domain of the protein. Briefly, SP-D was assayed in 96-well microtiter plates (Nunc labware products; MaxiSorp 96 well plates) coated with rabbit antirecombinant fragment human SP-D (α-rfhSP-D) at a 1:1,000 dilution as previously described and detected with biotinylated-α-rfhSP-D. Native human SP-D (0-500 ng/mL) was used as a standard (full methods are given in e-Appendix 1).[18]

SP-D Western Blotting

The same antibody, as described earlier, was used to detect "functional" SP-D in patient samples. A total of 20 μL of neat BAL or serum (100 μL of serum incubated with 20 μL of StrataClean Resin, in 500 μL of phosphate-buffered saline [Agilent Technologies, Inc] with calcium chloride 2 mM) was incubated for 30 min with rotation at room temperature. Samples were then spun at 1,300*g* and reduced according to the manufacturer's instructions (NuPAGE, Life Technologies). Proteins were resolved by 12% (w/v) sodium dodecyl sulfate-polyacrylamide gel electrophoresis (NuPAGE). Degradation of endogenous native human SP-D in BAL was visualized by immunoblotting of polyvinylidene difluoride membranes (iBlot, Life Technologies) using α-rfhSP-D antibodies.

Enzyme-Linked Immunosorbent Assay for Measures of Inflammation

Assays to measure BAL concentrations of myeloperoxidase (MPO, detection range 1.6-100 ng/mL) and neutrophil elastase (NE, detection range 0.4-25 ng/mL) were from Hycult Biotech. Eosinophilic cationic