

Basic fibroblast growth factor in asthma: Measurement in bronchoalveolar lavage fluid basally and following allergen challenge

Anthony E. Redington, MD,^a William R. Roche, MD,^b Jacqueline Madden, PhD,^a Anthony J. Frew, MD,^a Ratko Djukanovic, MD,^a Stephen T. Holgate, DSc,^a and Peter H. Howarth, MD^a *Southampton, United Kingdom*

Airway remodeling in asthma refers to a collection of chronic structural changes including subepithelial fibrosis, airway smooth muscle hypertrophy/hyperplasia, and possibly angiogenesis. The mechanisms leading to remodeling are not well defined. One molecule of possible relevance is basic fibroblast growth factor (bFGF), which is a potent mitogen for fibroblasts, airway smooth muscle cells, and endothelial cells. To test the hypothesis that bFGF expression is increased in asthma, we measured levels of the growth factor in bronchoalveolar lavage (BAL) fluid. Basally, BAL fluid bFGF concentrations were significantly higher in subjects with atopic asthma than in control subjects without asthma (median 0.22 vs 0.06 pg/mL, $P = .003$). The effect of acute allergen exposure was examined with a segmental bronchoprovocation model in a separate group of subjects with atopic asthma. Ten minutes after segmental bronchoprovocation there was a 5-fold increase in bFGF levels in BAL fluid recovered from allergen-challenged sites compared with control saline-challenged sites (1.52 vs 0.30 pg/mL, $P < .002$). We conclude that basal levels of BAL fluid bFGF are increased in atopic asthma and that a further increase occurs in response to acute allergen exposure. These findings lend support to the hypothesis that bFGF is implicated in airway remodeling in asthma. (J Allergy Clin Immunol 2001;107:384-7.)

Key words: Airway remodeling, basic fibroblast growth factor, bronchoalveolar lavage, bronchoscopy, fibrosis

Airway remodeling in asthma refers to certain structural changes that are present in the airway wall.¹ Particular attention has focused on airway smooth muscle hypertrophy and hyperplasia and on excess extracellular matrix (ECM) deposition and myofibroblast proliferation in the subepithelial region. There has also been recent interest in bronchial blood vessels, which are increased in

Abbreviations used

BAL:	Bronchoalveolar lavage
bFGF:	Basic fibroblast growth factor
ECM:	Extracellular matrix
FEV ₁ %:	Percent predicted FEV ₁
PC ₂₀ :	Provocative concentration of agonist producing a 20% fall in FEV ₁

number and size in asthma, suggesting that angiogenesis is a component of the response.² The mechanisms underlying these pathologic changes are poorly understood. One molecule of potential relevance is basic fibroblast growth factor (bFGF), which is a potent mitogen for many cell types including airway smooth muscle cells, fibroblasts, and endothelial cells.³

We hypothesized that bFGF is implicated in airway remodeling in asthma. To test this, we made comparative measurements of bFGF concentrations in bronchoalveolar lavage (BAL) fluid from subjects with and without atopic asthma. To investigate the release of bFGF in the airways in response to a physiologically relevant stimulus, we also made measurements in BAL fluid after segmental allergen bronchoprovocation in subjects with atopic asthma.

METHODS

Subjects

For basal measurements, we studied 7 mildly symptomatic subjects with atopic asthma (all men, aged 22.3 ± 1.0 years) and 8 control subjects without asthma (1 man and 7 women, aged 30.9 ± 5.0 years, 2 atopic). The subjects with asthma were all treated with short-acting β_2 -agonists as required, but none had received oral or inhaled corticosteroids for at least 3 months. Control subjects were taking no regular medication. To examine bFGF release, we performed segmental allergen bronchoprovocation in a separate group of 5 subjects with mildly symptomatic atopic asthma (2 men and 3 women, aged 24.4 ± 3.0 years). Two of these subjects were receiving inhaled corticosteroid therapy (beclomethasone dipropionate 1000 μ g and 400 μ g daily) but were asked to discontinue this 48 hours before bronchoscopy. The study was approved by the Southampton Joint University and Hospitals Ethics Committee. All subjects provided written informed consent.

From ^aUniversity Medicine and ^bUniversity Pathology, Southampton General Hospital.

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Reprint requests: A. E. Redington, MD, Department of Respiratory Medicine, 2nd Floor, Thomas Guy House, Guy's Hospital, London SE1 9RT, England, UK.

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Prebronchoscopy assessment

One to 2 weeks before bronchoscopy, subjects underwent measurement of FEV₁ and airway reactivity and skin prick testing. Subjects with asthma were asked to stop inhaled β_2 -agonists for at least 6 hours. The baseline FEV₁ was recorded with a wedge-bellows spirometer (Vitalograph Ltd, Buckingham, UK). Airway reactivity was measured as previously described⁴ with histamine (BDH, Lutterworth, UK) or methacholine (Sigma Chemical Company, Poole, UK). Atopy was defined as a positive response (wheal $\geq$ 3 mm at 15 minutes) to 1 or more of the following allergen extracts: *Der-matophagoides pteronyssinus*, cat allergen, mixed feathers, mixed grass pollens, and dog allergen (Miles Inc, Hollister Stier, Elkhart, Ind). For subjects undergoing segmental allergen bronchoprovocation, a skin prick test dose-response series was undertaken with serial 10-fold dilutions of the allergen that produced the largest response. The final concentration used for endobronchial instillation was 1/10 of the lowest that produced at least a 3-mm wheal.

Fiberoptic bronchoscopy and BAL

Bronchoscopy was performed as previously described.^{4,5} For basal measurements, the tip of the instrument was wedged into the anterior segment of the right upper lobe and 6 $\times$ 20-mL aliquots of 0.9% saline solution were instilled with recovery by suction after each instillation. Segmental bronchoprovocation was performed by instillation of 20 mL of 0.9% saline solution into the anterior segment of the right upper lobe and, after an interval of 20 seconds, the same volume of allergen solution into the medial segment of the middle lobe. Excess fluid was aspirated, and the 2 challenged segments were inspected to confirm airway narrowing at the allergen-challenged site. After 10 minutes, BAL was performed at both sites with 120 mL of 0.9% saline solution as above.

BAL fluid processing and differential leukocyte counts

BAL fluid was passed through a 100- μ m filter and then centrifuged at 4°C and 600g for 10 minutes. A protease inhibitor cocktail (composed of soybean trypsin inhibitor, 1 mg/mL; α 1 anti-trypsin, 1 mg/mL; pepstatin A, 1 mg/mL; EDTA, 2.5 mg/mL; benzamidine hydrochloride, 5 mg/mL; aprotinin, 1 mg/mL; and phenanthroline, 0.5 mg/mL; all Sigma Chemical Company) was then added at a final concentration of 1 μ L cocktail per 1 mL supernatant. Subsequently, BAL fluid supernatants were concentrated 10 $\times$ to 30 $\times$ with Centriprep 3000 concentrators (Amicon, Beverly, Mass). Centrifugation was performed at 4°C and 1300g for 90, 60, 30, and 10 minutes, and the low-molecular-weight fraction was decanted and discarded after each centrifugation. The concentrated supernatants were stored at -80°C.

The cell pellet was resuspended in PBS, and the total cell concentration was determined with a Neubauer hemocytometer (BDH, Dagenham, UK). The concentration was adjusted to 5 $\times$ 10⁵/mL, and cytocentrifuge slides were prepared with 100- μ L aliquots of cell suspension per slide (Cytospin; Shandon Scientific Ltd, Runcorn, UK). These preparations were air-dried, fixed in methanol, and stained with the Hema "Gurr" rapid staining set (BDH, Lutterworth, UK). Differential leukocyte counts were performed, counting 500 cells per slide.

bFGF ELISA

Levels of bFGF in BAL fluid concentrates were measured with a commercially available ELISA kit (R&D Systems, Minneapolis, Minn) according to the manufacturer's instructions. All assays were performed in duplicate. The limit of detection was 0.25 pg/mL. This assay is reported to show no cross-reactivity with human acidic FGF or other members of the FGF family.

Statistical analysis

Data for age, percent predicted FEV₁ (FEV₁%), and BAL fluid return volumes were expressed as mean $\pm$ SEM. Because bFGF levels were not normally distributed, values were log transformed before statistical analysis. Comparisons were then undertaken with unpaired and paired *t* tests as appropriate. Differential leukocyte counts were expressed as median and range and compared with the Mann Whitney *U* and Wilcoxon tests. Correlations were sought with the Pearson and Spearman tests for normally and nonnormally distributed data, respectively. A *P* value < .05 was considered statistically significant. Statistical analysis was performed with StatView 4.02 for Macintosh (Abacus Concepts, Berkeley, Calif).

RESULTS

Subject comparisons

Control subjects and subjects with asthma undergoing basal measurements did not differ significantly in age (*P* = .11), but FEV₁% measurements were significantly lower for the subjects with asthma (85.2% $\pm$ 3.9% vs 103.6% $\pm$ 2.9%, *P* = .002). The subjects with asthma in this part of the study had varying degrees of airway hyperreactivity (geometric mean PC₂₀ [provocative concentration of agonist producing a 20% fall in FEV₁] methacholine, 0.88 mg/mL; range, 0.31-8.34 mg/mL), whereas the control subjects all had PC₂₀ values falling outside the asthmatic range (>16 mg/mL). Subjects with asthma undergoing endobronchial allergen challenge had milder disease than those receiving only baseline BAL, in terms of both FEV₁% (115.5% $\pm$ 5.1%) and airway reactivity (geometric mean PC₂₀ histamine, 3.30 mg/mL; range, 2.21-4.67 mg/mL).

BAL fluid parameters

For subjects undergoing basal measurements, BAL fluid return volumes did not differ significantly between subjects with asthma and control subjects (60.3 $\pm$ 3.8 mL vs 70.9 $\pm$ 8.1 mL, *P* = .28). The proportion of eosinophils was significantly higher in the subjects with asthma than in the control subjects (1.3% [0.2%-2.6%] vs 0.2% [0%-0.6%], *P* < .05), but the 2 groups did not differ significantly with regard to other leukocytes (data not shown). There was an inverse correlation between FEV₁% and the percentage of BAL lymphocytes in the subjects with asthma (*r* = -0.89, *P* < .05), but no other correlation with physiologic parameters was found.

After segmental bronchoprovocation, marked airway narrowing (estimated visually as $\geq$ 75% reduction from baseline) was evident within a few minutes at the allergen-challenged, but not the saline-challenged, segment. Return volumes did not differ significantly between the 2 sites (74.0 $\pm$ 8.2 mL vs 61.7 $\pm$ 7.7 mL, *P* = .19), and there were also no significant differences in BAL leukocyte counts (data not shown).

Immunoreactive bFGF in BAL fluid

Measured bFGF levels were corrected for the concentration factor so as to express levels of bFGF in unconcentrated BAL fluid. Basal bFGF concentrations were significantly higher in the subjects with asthma than in the control subjects (median, 0.22 vs 0.06 pg/mL; *P* = .003) (Fig 1).

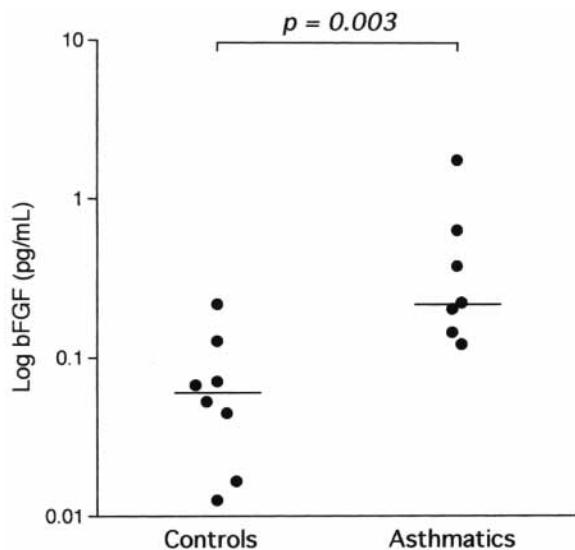


FIG 1. Comparison of BAL fluid bFGF concentrations in subjects with asthma ($n = 7$) and control subjects ($n = 8$). Horizontal bars represent median values. Statistical analysis was performed with the Mann-Whitney U test on log-transformed data.

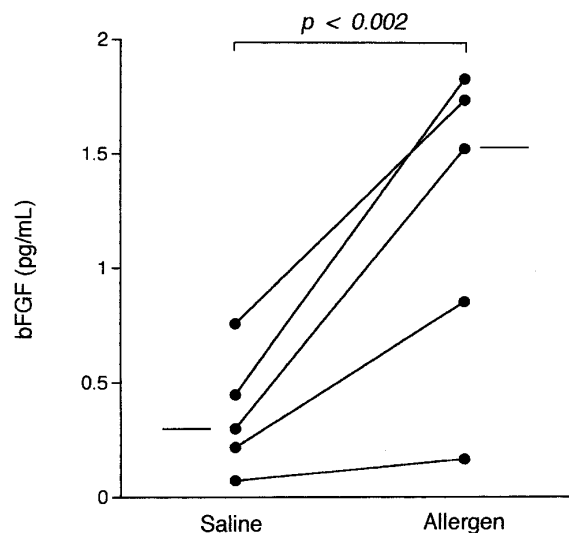


FIG 2. Comparison of bFGF concentrations between saline-challenged and allergen-challenged segments in subjects with asthma ($n = 5$). Horizontal bars represent median values. Statistical analysis was performed with the Wilcoxon signed rank test.

There were no significant correlations between bFGF concentrations and either physiologic indexes or BAL leukocyte counts. After segmental bronchoprovocation, the concentration of bFGF was greater in each individual at the allergen-challenged site than at the saline-challenged site (median, 1.52 vs 0.30 pg/mL; $P < .002$) (Fig 2).

DISCUSSION

In this study we have shown that basal levels of bFGF in BAL fluid are increased in atopic asthma and that a further increase occurs in response to allergen exposure. To our knowledge this report represents the first description of the *in vivo* expression of this growth factor in asthma.

One potential explanation for the rapid rise in BAL fluid bFGF after segmental allergen challenge would be release of the growth factor from mast cells. The immediate airway response to allergen is considered to result principally from IgE-dependent mast cell activation. Using similar methods to those described here, other investigators have reported the release of various mast cell-derived substances at early time points.⁶ Human lung mast cells can certainly express bFGF, and despite the absence of a classic signal peptide, it appears to be localized at the subcellular level to mast cell granules.⁷ Although IgE-dependent release has not yet been described, such a mechanism could provide a plausible explanation for our findings. Other cells, in particular airway epithelial cells, likely represent additional cellular sources of bFGF in the airways. Airway epithelial cells can express functionally active IgE receptors⁸ and thus could conceivably also contribute to bFGF release.

Another possibility to explain the rapid rise in BAL fluid bFGF after allergen exposure would be release of ECM-bound growth factor. Immunohistochemical studies in a variety of tissues have suggested that bFGF is stored extracellularly bound to basement membrane heparan sulfate proteoglycans.⁹ Release from these storage sites could be induced by mast cell degranulation products, especially heparin, which represents a major mediator released after IgE-dependent mast cell activation. Preparations of heparin are generally considered to bind bFGF at least as strongly as heparan sulfate, and indeed heparin and related oligosaccharides can induce the rapid release of bFGF *in vitro* from ECM secreted by cultured endothelial cells and from bovine corneal Descemet's membrane.¹⁰ On this basis, mast cell-derived heparin might competitively elute the growth factor from its matrix-bound state, resulting in increased levels detectable in BAL fluid.

In this study, we also found higher basal levels of bFGF in subjects with asthma than in healthy control subjects. This finding could reflect increased synthesis or release, either from resident stromal cells or infiltrating inflammatory cells, as well as reduced degradation. Alterations in the degree of extracellular binding might also be a factor leading to elevated BAL fluid bFGF levels. Mast cell activation is evident in chronic asthma, with electron microscopic appearances showing mast cell degranulation and increased quantities of mast cell products recoverable in BAL fluid.⁶ In addition to acting as a cellular source of bFGF, therefore, mast cells might, by releasing heparin, alter the distribution of bFGF between ECM binding sites and the soluble phase.

Two potential limitations of this study are the relatively small numbers of subjects studied and the sex imbal-

ance between the subjects with asthma and control subjects. However, taken together, our findings do provide preliminary support for the hypothesis that this growth factor is important in airway remodeling in asthma. Further studies using additional methods will be required to define the role of bFGF more precisely.

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