Bronchial Leukocyte Proteinase Inhibitor Levels in Bronchial Washings in Asthma Patients*

Jan J. Ochnio, M.D.;† Raja T. Abboud, M.D., F.C.C.P.; Stephen Lam, M.D., F.C.C.P.; Shivraj S. Johal, M.Sc.; Craig E. Smith, Ph.D.; and David A. Johnson, Ph.D.

To evaluate whether epithelial damage of airways in asthma could be related to diminished levels of the low molecular weight bronchial leukocyte proteinase inhibitor (BLPI) in airways, we determined BLPI in bronchial washings of 13 asthma patients and 13 healthy subjects, using a sensitive enzyme-linked immunoassay. The patients had asthma due to western red cedar and had bronchial washings done 24 to 48 hours after a mild to moderate asthmatic reaction induced by inhalation challenge. We did not find significant differences in BLPI concentrations in lavage fluid of asthma patients and healthy control subjects. The ratios of BLPI to albumin levels in bronchial washings appeared to be lower among asthmatic patients, but this difference was mainly due to an increase in albumin levels in lavage fluid in asthma. In addition, there were no significant differences in BLPI levels in washings obtained from main and segmental bronchi in both control subjects and asthma patients.

It is known that epithelial damage may be present in the airways of patients with asthma.1,3 Epithelial airway damage may be a mechanism aggravating asthma by exposing nerve endings to stimuli leading to increased bronchoconstriction via the vagal reflex,4 as well as by enhancing penetration of antigenic or irritating material from the airway lumen to the subepithelial tissues.1

Epithelial damage in asthma may be potentially due to several mechanisms. Based on observations that eosinophilic major basic protein can damage guinea pig tracheal mucosa5 and that eosinophils and eosinophilic basic protein are increased in tissues and sputum of asthmatic patients,6,7 it was suggested that eosinophilic basic protein released from eosinophils may lead to epithelial airway damage.8 The presence of mast cells in damaged areas suggests their possible role in airway damage by release of histamine and other inflammatory mediators.3 Elastase and other proteolytic enzymes released from neutrophils,8 which may be increased in the airways in asthma,9,10 or from mast cells, which themselves contain elastase and cathepsin G-like enzymes,11 may also play a role in epithelial airway damage.

The major proteinase inhibitor protecting the bronchial epithelium against proteolytic injury is the low molecular weight bronchial proteinase inhibitor (BLPI).12 This inhibitor has been localized by immunohistochemical techniques to the serous glands of the bronchial epithelium13,14 and to the nonciliated epithelial cells of the bronchioles.15 Impaired secretion of BLPI could be a factor enhancing bronchial epithelial damage in asthma by decreasing protection against proteolytic enzymes.

The purpose of our study was to evaluate whether there is a decrease in BLPI in the airways in asthma by comparing BLPI levels in bronchial washings from patients with asthma with BLPI levels in healthy subjects.

**Materials and Methods**

**Subjects Studied**

We studied 13 healthy subjects (mean age, 30±9 years) and 13 patients (mean age, 36±8 years) with confirmed occupational asthma due to western red cedar.14 The normal individuals had no respiratory symptom or recent respiratory infection and had normal results of spirometric evaluation. All subjects studied, both control subjects and asthma patients, were nonsmokers and were part of a study reported previously.17 Patients with asthma underwent bronchoscopy 24 to 48 hours following a positive bronchial reaction to inhalation challenge with plicatic acid, the chemical in red cedar responsible for the development of red cedar asthma.16 The procedures were approved by the University Ethics Committee on Human Experimentation, and informed consent was obtained from all subjects prior to their participation.

**Methods**

Bronchial lavage was performed through a fiberoptic bronchoscope as described in detail previously.17 Following premedication with atropine and local anesthesia with lidocaine (Xylocaine). Asthmatic patients inhaled 200 µg of salbutamol (albuterol) from a metered-dose inhaler 15 minutes prior to bronchoscopy. The lavage...
of a main bronchus was performed by instillation of 10 ml of saline solution through the bronchoscope with its tip in the main bronchus just distal to the carina, followed by immediate suction of the lavage fluid. For segmental lavage, the bronchoscope was wedged into a segmental or subsegmental bronchus, and 10 or 20 ml of saline solution was instilled as a single bolus and immediately suctioned. After a cell count and differential were obtained, the recovered fluid was centrifuged at 500 g for 15 minutes, and the supernatants were kept frozen in small aliquots at −70°C until assayed. Albumin was quantitated in uncentrifugated fluid by radial immunodiffusion using low-level plates (Behring Diagnostics); the lower limit of the assay was 5 μg/ml.

BLPI in the uncentrifugated bronchial lavage fluid was quantified by slight modifications of the double antibody sandwich enzyme linked immunosorbent assay (ELISA) described by Kramps and coworkers. Briefly, the samples were treated with perchloric acid to precipitate proteins and dissociate any BLPI-protease complexes, and the supernatant was neutralized with potassium hydroxide (KOH) and separated by centrifugation. The treated lavage fluid was then diluted in phosphate-buffered saline solution containing non-immune rabbit plasma and then added to the wells of an ELISA microplate (Immulon I, Dynatech Lab, Inc), precoated with rabbit anti-BLPI antibody (IgG fraction). After incubation and washing, BLPI bound to the plate was detected by its binding of rabbit anti-BLPI IgG linked to alkaline phosphatase. The activity of the bound enzyme was then determined using alkaline phosphatase substrate (Sigma Chemical Co) and read with an automated microplate reader (Flow Laboratories, Inc). Each sample was assayed at two dilutions, with each dilution applied to three wells in the micro-ELISA plate; the results of the two dilutions agreed to within 10 percent. The range of our ELISA assay was from 1 to 6 ng of BLPI/ml. Standards of pure BLPI, prepared from spumus by the method of Smith and Johnson, were included in each plate. Antibody to BLPI was prepared by immunizing rabbits with pure antigen; the IgG fraction was isolated by protein A-Sepharose chromatography (Pharmacia Corp).

To determine the presence of free elastase activity in bronchial washings, we used a specific and sensitive substrate to assay for elastase, methoxy succinyl-alanyl-alanyl-prolyl-valyl-p-nitroanilide (Sigma Chemical Co). Sufficient amounts of bronchial washings were available for elastase assay from six asthmatic patients; in one patient only a sample of main bronchial washings was assayed, in three patients segmental bronchial washings were tested, while in two patients samples from both main and segmental bronchi were assayed. For each sample, duplicate assays were performed by incubating 100 μl of sample with 1 ml of 0.4 mM substrate in 0.2 M TRIS, 1 M NaCl, pH 8.0, at 37°C for 3 hours. Elastase activity was determined from the change in optical density at 410 nm, using standards of human neutrophil elastase purified in our laboratory, and appropriate blanks. The assay was able to detect elastase activity equivalent to about 2.5 ng of human neutrophil elastase.

Statistical Analysis

To evaluate the significance of differences in results between asthmatic and control subjects, a nonparametric test, the Wilcoxon sum rank test, was used because the results were not normally distributed. For comparison of results of albumin levels, which were normally distributed, the unpaired t test was used.

RESULTS

There were seven samples of lavage from a main bronchus available for BLPI assay from the asthma patients and seven from the control subjects. The samples from the segmental bronchial lavages were divided into two groups according to whether 10 or 20 ml of saline solution was used for lavage. For the segmental lavages done with 10 ml of saline solution samples were available from ten asthmatic patients and from 11 control subjects, while for the lavages done with 20 ml of saline solution seven samples were available from the asthma patients and seven from the control subjects.

BLPI concentrations in bronchial lavage fluid, expressed as μg BLPI/ml of recovered lavage fluid, are shown in Figure 1 and Table 1. Although in some asthma patients there was a tendency toward lower levels than in control subjects, both for main bronchial and segmental lavage samples, the differences were not statistically significant. The levels of BLPI in lavage samples from the main bronchus were similar to those

![Figure 1. BLPI levels in bronchial lavage fluid expressed as μg BLPI/ml of recovered fluid in asthmatic subjects and controls. The bronchus lavaged and the volume used are shown on the X-axis. Bars indicate median values. There were no statistically significant differences between controls and asthma patients.](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21577/)
obtained from lavage of a segmental bronchus using either 10- or 20-ml lavage volumes in both asthma patients and control subjects.

Albumin levels in lavage fluid from a main bronchus (Table 1) were significantly higher in asthmatic than in control subjects (p<0.01). Although albumin levels in the 10 ml and 20 ml lavages from segmental bronchi tended to be higher in asthmatic than control subjects, the differences were not statistically significant. BLPI levels expressed as μg BLPI/μg albumin are shown in Figure 2. Levels in main bronchial lavage and in the 20-ml segmental lavage were lower in asthmatic than in control subjects (p<0.02 and p<0.05, respectively). BLPI-to-albumin ratios in the 10-ml segmental lavage were not significantly different in asthmatic and control subjects.

No elastase activity was detected in seven of the eight samples of bronchial washings obtained from six patients with asthma. In one patient, trace amounts of elastase activity equivalent to about 20 ng/ml were detected in segmental bronchial washing but not in the washings from the main bronchus.

**DISCUSSION**

Bronchial leukocyte proteinase inhibitor has anti-protease activity against human leukocyte elastase, cathepsin G as well as other proteases.25-27 However, its main physiologic role in vivo is thought to be inhibition of human leukocyte elastase, against which it has a high kinetic association constant.20,28 BLPI accounts for 75 to 90 percent of the antiprotease activity of bronchial lavage fluid,12,29 only about 15 percent of the total amount of BLPI in bronchial lavage fluid was found complexed with proteases in healthy persons.12

In this study we found no significant differences in BLPI levels between the main and segmental bronchi, suggesting that BLPI is secreted evenly in large and small bronchi, at least from the second generation (main bronchi) to fourth and fifth generations (segmental bronchi) and even higher up in the airway tree. As such, the bronchial lavage fluid from these patients is an excellent model for studying the effects of BLPI on protease activity in vivo.
tal and subsegmental bronchi). We sought to determine whether there were differences in BLPI levels in bronchial secretions in patients with red cedar asthma compared with healthy control subjects. The ideal approach would have been to collect undiluted bronchial secretions for assay, but such an approach is not feasible in volunteers. We used instillation of small volumes of saline solution through the fiberoptic bronchoscope followed by immediate aspiration of the instilled fluid to recover bronchial secretions. BI levels in the recovered fluid may be affected by variability in the dilution and recovery of bronchial secretions. However, it is unlikely that there was a sufficient discrepancy in the relative recovery of bronchial secretions in the two groups to affect our conclusions significantly, since the proportion of instilled fluid recovered and cell counts in the fluid were similar in asthma patients and control subjects.17

We did not find any significant differences in the levels of BLPI in bronchial lavage fluids between asthma patients and control subjects when levels were expressed in μg/ml of lavage fluid. However, BLPI levels expressed per μg of albumin in lavage fluid appeared to be decreased in asthma. This difference is apparently due to the significant increase in the amount of albumin in bronchial lavage fluid in asthma, as has been previously reported,17 supporting the previous finding that, in inflammatory conditions where albumin levels may be increased in the bronchial lining fluid, normalizing bronchial lavage constituents for the level of albumin may not be a valid way of comparing values in disease and health.

Although BLPI levels in bronchial lavage in our subjects with asthma were not decreased, this does not exclude the possibility that protease-antiprotease imbalance may be contributing to epithelial damage in more severe asthma. It should be noted that the assay used in this study determined both free BLPI and BLPI complexed with proteases, as a result of the initial treatment of the sample with perchloric acid which dissociates BLPI protease complexes.25 However, it is unlikely that BLPI present in bronchial lavage in our asthma patients could have been fully complexed with proteases. If that were the case, one would expect free proteolytic activity in the lavage fluid similar to the findings in secretions from patients with chronic bronchitis.25,26 We did not have sufficient bronchial washings available to determine the functional activity of BLPI in our samples. However, we assayed for the presence of elastolytic activity in eight bronchial washings from six patients and found no activity in seven of the eight samples. It should be noted that the patients we studied generally had mild asthma whose disease was occupational due to western red cedar and who had undergone lavage 24 to 48 hours after a mild to moderate asthmatic reaction was induced with bronchial inhalation challenge. There was only minimal or no increase in the number of neutrophils in lavage fluid in these patients.17 With more severe and prolonged asthma, the increased inflammatory reaction and the release of proteolytic enzymes from neutrophils, as well as elastase and cathepsin G-like enzymes from mast cells,11 may saturate the antiprotease protection of BLPI and thus leave the airway epithelium susceptible to proteolytic injury.

REFERENCES
5. Frigas E, Loegering DA, Gleich GJ. Cytotoxic effects of the guinea pig eosinophil major basic protein on tracheal epithelium. Lab Invest 1980; 42:35-43
6. Frigas E, Loegering DA, Solley GO, Farrow GM, Gleich GJ. Elevated levels of the eosinophil granule major basic protein in the sputum of patients with bronchial asthma. Mayo Clin Proc 1981; 56:345-53
7. Filley WV, Holley KE, Kephar GM, Gleich GJ. Identification by immunofluorescence of eosinophil granule major basic protein in lung tissues of patients with bronchial asthma. Lancet 1982; 2:11-15
16. Chan-Young M, Lam S, Koerner S. Clinical features and natural history of occupational asthma due to Western Red Cedar (Thuja

CHEST / 93 / 5 / MAY, 1988
1012

Bronchial Leukocyte Proteinase Levels in Asthma (Ochnio et al)