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Bronchial brush biopsies for studies of epithelial inflammation in stable asthma and nonobstructive chronic bronchitis

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ABSTRACT: Recently, bronchial brush biopsy (BBB) has been introduced as a complimentary method to bronchial forceps biopsy for the study of bronchial epithelial cells. We wanted to determine whether epithelial inflammatory cells in bronchial brush biopsies can reflect mucosal inflammation assessed indirectly by levels of cellular activation markers in bronchial lavage fluid.

We studied 15 healthy controls, 11 asthmatics with regular steroid inhalation therapy, 13 asthmatics without steroids, and 10 smokers with nonobstructive chronic bronchitis. Differential counts of epithelial and inflammatory cells were made from the BBB material. Bronchial lavage levels of eosinophil cationic protein (ECP), myeloperoxidase (MPO), tryptase, hyaluronan and interleukin-8 (IL-8) were measured as indirect markers for inflammatory cell activation.

We found an increased percentage of eosinophil granulocytes in the BBB from the steroid-untreated asthmatic patients (1.16%) in comparison to the other groups (0.11%, 0.09% and 0.02%, respectively; $p<0.01$). In the steroid-untreated asthmatic patients, the percentage of eosinophils correlated with ECP in bronchial lavage fluid ($r=0.73$; $p<0.01$), indicating that the BBB method can reflect the degree of eosinophilic activation. A negative correlation was found for the percentage of eosinophils in BBB with levels of provocative concentration of methacholine causing a 20% fall in forced expiratory volume in one second (PC_{20}) for the asthmatic patients in the study ($r= -0.67$; $p<0.003$).

The bronchial brush biopsy method appears to give information on the changes present in superficial bronchial epithelium in inflammatory airways disease. These changes appear to relate to the degree of inflammatory activity and disease severity in asthma.

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The flexible fibreoptic bronchoscope has proved to be a useful research tool in investigating inflammatory airway diseases. Recently, bronchial brush biopsy (BBB) has been introduced as a complimentary method to bronchial forceps biopsy for the study of bronchial epithelial cells both morphologically [1, 2] and functionally [3, 4]. The method gives information specific to the superficial airway epithelium, since principally cells above the basement membrane are collected. The cytological outcome for the BBB method is dominated by >90% epithelial cells, but inflammatory cells have also been demonstrated [1, 2].

In asthma, biopsies obtained using bronchial forceps have shown the mucosal airway changes to consist of a predominance of eosinophil and mast cell infiltration [5–9]. Specific molecules from activated inflammatory cells have been detected in BAL from asthmatic patients, including eosinophil cationic protein (ECP) from eosinophil granulocytes [10], tryptase from mast cells [11], and free hyaluronan from fibroblasts [12]. Regular treatment with inhaled steroids has been shown to ameliorate the airway inflammation in asthma [13–15].

The airway inflammation in chronic bronchitis has not been as well characterized as that in bronchial asthma. Histopathological studies of large airway mucosa in patients with chronic bronchitis have demonstrated infiltration of mononuclear phagocytes [16], lymphocyte infiltration and lymphocyte activation [17]. BAL and bronchial lavage fluids have shown elevated numbers of macrophages and neutrophil granulocytes [18, 19], as well as increased levels of myeloperoxidase (MPO) [20].

We attempted to investigate the usefulness of the BBB method in characterizing the bronchial epithelial inflammation in three groups of well-defined patients: asthmatics with and without inhaled steroid therapy, and patients with nonobstructive chronic bronchitis. We wanted to determine whether accumulation of epithelial inflammatory cells in bronchial brush biopsies can reflect intrabronchial mucosal inflammation assessed indirectly by levels of activation markers for airway inflammatory cells.

Materials and methods

Design

Flexible fibreoptic bronchoscopy was performed, and standardized bronchial brush biopsies as well as small volume (20 mL) lavage of the large airways were obtained. Differential counts of epithelial cells, eosinophil granulocytes (Eos), polymorphonuclear granulocytes (PMN), macrophages and mononuclear cells were made from the BBB material. Bronchial lavage fluid and serum levels of ECP, MPO, tryptase and hyaluronan were used as indirect markers for activation of eosinophils, neutrophils, mast cells and fibroblasts, respectively. Interleukin-8 (IL-8) was measured as a marker for chemotactic attraction and activation of neutrophils.

Atopy was defined as a history of upper or lower airway symptoms to common aeroallergens verified by positive skin prick test (birch tree, grass and mugwort pollen, dog and cat epidermis, *Aspergillus fumigatus*, *Dermatophagoides pteronyssinus*) or by radioallergosorbent test for specific immunoglobulin E (IgE) (Phadiatop®; Pharmacia, Uppsala, Sweden). All bronchoscopies were performed out of the pollen allergic season and by the same experienced bronchoscopist (GCR).

Subjects

Group 1. Fifteen healthy controls (14 lifelong nonsmoking volunteers and one ex-smoker since 20 yrs). All had normal spirometry, negative methacholine test (provocative concentration causing >20% fall from baseline forced expiratory volume in one second (FEV_1) (PC_{20} >16 $mg \cdot mL^{-1}$), and no signs of infectious respiratory disease during the previous 4 weeks. Four of these subjects were atopic.

Group 2. Twenty four patients with bronchial asthma defined according to the criteria of the American Thoracic Society (ATS) [21]. They all had positive methacholine challenge test (PC_{20} <4 $mg \cdot mL^{-1}$), or an increase in FEV_1 of >20% on β_2-agonist inhalation. All were lifelong nonsmokers (except for one ex-smoker), with no signs of infectious respiratory disease or obstructive symptoms during the previous 4 weeks. These patients were divided into two subgroups.

Group 2a. Thirteen patients with mild asthma requiring symptomatic therapy with inhaled β_2-agonists only occasionally. None used inhaled or oral steroid medication, nor other oral antiasthma medication. Nine of these subjects were atopic.

Group 2b. Eleven patients with bronchial asthma and on a regular medication with inhaled steroids administered by powder inhaler devices for more than 1 yr (mean 836 µg beclomethasone or budesonide per day), together with inhaled β_2-agonist medication as required. Seven of these subjects were atopic.

Group 3. Ten patients with nonobstructive chronic bronchitis. All were current smokers (mean 34 pack-years) with productive daily cough for at least three consecutive months each year during the past 2 yrs, and they had normal spirometry (table 1). Methacholine tests were negative (PC_{20} >16 $mg \cdot mL^{-1}$), and no increase was seen in FEV_1 on β_2-agonist inhalation. None had a history of recurrent infective exacerbations. Three of these subjects were atopic. For further patient data see table 1.

Of the 24 asthmatic patients, four used oral β_2-agonists regularly, and one used oral theophylline preparations (all in Group 2b). Of the 10 patients with chronic bronchitis, one used ipratropium bromide for inhalation as required, the others used no airway medication.

The duration of the respiratory symptoms varied for the asthmatics between 3 to 30 yrs (mean 9 yrs), and for the bronchitics between 2 to 20 yrs (mean 8.5 yrs). Ventilatory lung function (FEV_1) was measured with a Bernstein spirometer or a Vitalograph. All methacholine provocation tests were performed within 4 weeks prior to the bronchoscopy.

Criteria for exclusion were: abnormal chest radiograph; bronchial hypersecretion caused by factors other than cigarette smoking for the bronchitis patients; age >65 yrs; and, for safety reasons, a methacholine test of <0.5 $mg \cdot mL^{-1}$ in the steroid untreated asthmatic patients (Group 2a). The study design was approved by the Ethics Committee of the University of Göteborg, and all subjects gave their consent after both written and oral information.

Fibreoptic bronchoscopy

Premedication was given with diazepam 5 mg orally, followed by 0.5–1 mL morphine-scopolamine *i.m.* For

Table 1. – Clinical details of subjects: gender, number of subjects with atopy, age, FEV1 and level of positive methacholine provocation test (PC_{20})

Group		Sex M/F	Atopy n	Age yrs	FEV_1 % pred	PC_{20}# $mg \cdot mL^{-1}$
1.	Healthy controls (n=15)	4/11	4	39 (20–62)	106 (130–85)	>16.0
2a.	Asthma without inhaled steroids (n=13)	3/10	9	40 (22–62)	92 (80–103)	0.75 (0.5–2.0)
2b.	Asthma with inhaled steroids (n=11)	7/4	7	47 (22–63)	93 (83–101)	4.00 (2.0–8.0)
3.	Chronic bronchitis (n=10)	4/6	3	45 (31–62)	100 (83–113)	>16.0

Values are presented as mean, and range in parenthesis. #: geometric mean. M: male; F: female; FEV_1: forced expiratory volume in one second; PC_{20}: provocative concentration of methacholine causing a 20% fall in FEV_1; % pred: percentage of predicted normal.

topical anaesthesia, 1% preservative-free tetracaine was sprayed with a Devilbiss nebulizer in the larynx, and additional anaesthesia was applied through the bronchoscope channel for the lower respiratory tract. Bronchoscopy was performed transorally, and various models of Olympus flexible fibreoptic bronchoscopes were used. Throughout the procedure, all subjects in the study, including the healthy controls, received continuous *i.v.* infusion of terbutaline at 2.5 $\mu g \cdot min^{-1}$. Supplemental 100% oxygen was delivered nasally at 2–3 $L \cdot min^{-1}$, and blood oxygen saturation was monitored with an Ohmeda pulse oximeter (Ohmeda, Louisville, KY, USA). Seven asthmatic patients, all from the group without regular steroid medication (Group 2a), developed mild obstruction during the latter part of the bronchoscopy. Only one of these required additional bronchodilator therapy for the symptoms to recede. None developed a late asthmatic reaction after the investigation.

Collection of samples

All samples were collected between 8.30 and 10 a.m. Serum was separated from the 10 mL venous blood sample according to AHLSTEDT *et al*. [22], and frozen at -20°C for later analysis of ECP, MPO, IL-8, hyaluronan and tryptase.

Bronchial lavage fluid was obtained by a single instillation of 20 mL sterile phosphate-buffered saline (PBS), with the bronchoscope in an unwedged position in the anterior segment of the right upper lobe. The fluid was immediately aspirated, collected in a sterile container and transported on ice to the laboratory, where the cells were sedimented by centrifugation and the supernatant frozen at -20°C for later analysis of inflammatory markers.

Standardized bronchial brush biopsies were then taken with a sterile single-sheathed nylon cytology brush (Olympus BC 9C-26101) as described previously [1]. In brief, three consecutive brushings from an approximately 3–4 cm^2 intrabronchial area were taken from the proximal part of the right main bronchus. The brush was agitated in a vortex in 1 mL 0.9% saline between each brushing, and cytocentrifuge slides (Shandon Southern Products Ltd, Runcorn, UK) were then made from 100 µL aliquots of the pooled brush samples. Slides were immediately fixed in 96% alcohol and then stained with May-Grünwald Giemsa for later identification of cell types on a morphological basis. Percentages of epithelial cells, eosinophil granulocytes, polymorphonuclear granulocytes, macrophages and mononuclear cells were calculated by counting 1,000 cells using a standard light microscope at × 100 magnification. The term "mononuclear cells" was used, since no further differentiation between lymphocytes and monocytes was attempted. All samples were analysed in a blinded manner.

Analysis of inflammatory markers

Kits for analysis of ECP [23], MPO [24], hyaluronan [25] and tryptase [26] (Pharmacia Diagnostics AB, Uppsala, Sweden) were used according to the instructions of the manufacturers.

IL-8 was analysed using a modified sandwich fluorescent enzyme immunoassay method (Pharmacia Diagnostics AB, Uppsala, Sweden). Briefly, the bronchial lavage fluid was incubated in microtitre wells coated with a mouse monoclonal antibody against IL-8. All samples were diluted in the standard dilution buffer. After washing, a goat anti-IL-8 β-galactosidase-conjugate was added and the plates were incubated. The response was measured fluorometrically. Recombinant IL-8 [27] was used as standard ranging 10–2,000 $ng \cdot L^{-1}$. The intra- and interassay coefficients of variation were less than 10% and the detection limit was 5 $ng \cdot L^{-1}$.

Statistical evaluation

The inflammatory cell counts are expressed as arithmetic mean and standard deviations (SD). The levels of soluble markers are presented as median and SEM. Non-parametric statistical analysis was used throughout the study since each subject group was small, and the data were without normal distribution. Kruskall-Wallis test was used to analyse the significance of differences in numerical data between the four groups of subjects. Mann-Whitney U-test was used for the comparison of the distribution of inflammatory markers and bronchial epithelial cells between subgroups of subjects. Spearman's rank correlation test was used to examine possible associations between markers, cells and clinical parameters.

Results

Inflammatory cells in bronchial brush biopsies

The percentage of eosinophil granulocytes was significantly higher in the steroid-untreated asthmatic patients (Group 2a) ($p < 0.01$) than in the controls (fig. 1). In contrast, the percentage of epithelial cells, neutrophils, macrophages and mononuclear cells did not differ between the groups (table 2).

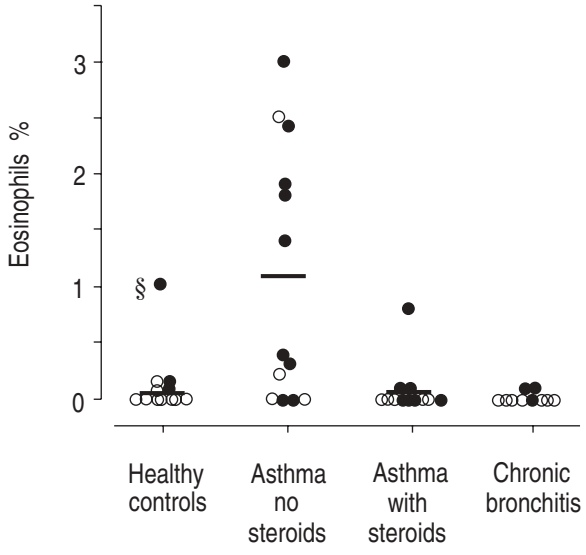

Fig. 1. – Percentage of eosinophils in bronchial brush biopsies in patients and healthy controls. Subjects with atopy are indicated by filled circles. Bold lines represent mean values. §: atopic control, who developed asthma 1 yr after the study.

Table 2. – Different counts of cells in the bronchial brush biopsies

Group		Epi %	Eos %	PMN %	Mac %	Mono %	Others %
1.	Healthy controls	91.2 (1.1)	0.11 (0.07)	2.33 (0.8)	0.97 (1.2)	0.99 (0.2)	4.6 (0.3)
2a.	Asthma without inhaled steroids	91.3 (1.0)	1.16 (0.3)**	1.52 (0.7)	0.36 (0.4)	1.67 (0.1)	4.00 (0.3)
2b.	Asthma with inhaled steroids	92.4 (1.9)	0.09 (0.07)	2.43 (1.7)	0.25 (0.2)	1.01 (0.1)	3.76 (0.5)
3.	Chronic bronchitis	91.4 (1.9)	0.02 (0.01)	2.13 (1.2)	0.27 (0.5)	1.52 (0.3)	4.57 (0.5)

Values are presented as mean and SEM in parenthesis. Epi: epithelial cells; Eos: eosinophils; PMN: polymononuclear granulocytes; Mac: macrophages; Mono: mononuclear cells. **: p<0.01 *versus* the controls (Mann-Whitney U-test).

The percentage of ciliated epithelial cells for all subjects were 58% (SEM 2%), goblet cells 10% (SEM 2%), and basal cells (including epithelial cells of uncertain morphology) 32% (SEM 1%). No differences were found between the groups.

The 23 atopic subjects from Groups 1–3 had a higher mean percentage of eosinophils in bronchial brush biopsies (mean 0.58, SEM 0.4) compared with the 26 nonatopic subjects (mean 0.15, SEM 0.1), this difference was not statistically significant (p=0.06).

In the asthmatic patients (Group 2) the percentage of eosinophils in brush biopsies showed a significant negative correlation with levels of PC_{20} (fig. 2), (r= -0.67; p<0.003). This association was present for the steroid-untreated asthmatic patients (Group 2a), (r= -0.77; p<0.02) but not for the steroid treated asthmatics (Group 2b), (r=0.14; NS).

Correlations between bronchial brush biopsies and inflammatory markers

The percentage of eosinophils in brush biopsies for the steroid-untreated asthmatic patients (Group 2a) showed a high correlation with bronchial lavage levels of ECP (r=0.73; p<0.01) (fig. 3): a weaker correlation was found when all subjects in the study were analysed (r=0.31; p<0.05).

Weak correlations were found for the percentage of neutrophils with bronchial lavage levels of MPO (r=0.33; p<0.05) and IL-8 (r=0.39; p<0.01) when all subjects were analysed. The percentage of mononuclear cells and macrophages did not correlate significantly with markers in bronchial lavage (data not shown).

No significant correlations were found for the percentage of inflammatory cells in bronchial brush biopsies and the levels of inflammatory markers in serum.

Inflammatory markers in bronchial lavage and in serum

The asthmatic patients not given inhaled steroid therapy (Group 2a) had significantly higher mean bronchial lavage levels of ECP (p<0.02) and hyaluronan (p<0.002) compared with the controls (table 3): they also had significantly higher serum levels of ECP (p<0.05), (table 4).

The asthmatic patients with regular inhaled steroid therapy (Group 2b) had significantly higher mean bronchial lavage levels of ECP (p<0.02) and hyaluronan (p<0.05) compared with the healthy controls (table 3). Their serum levels, however, did not differ (table 4).

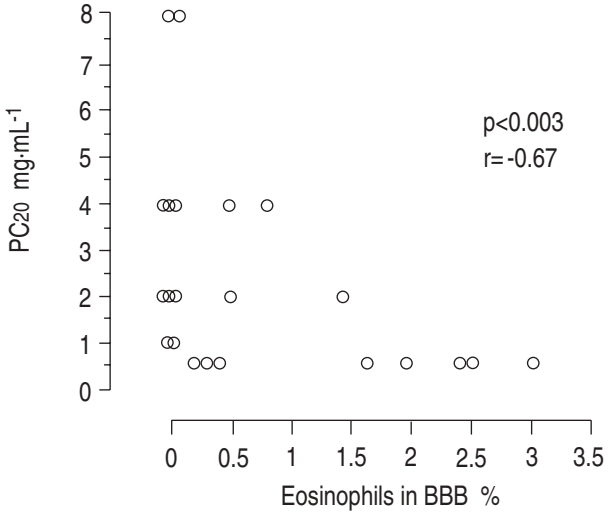

Fig. 2. – Correlation between level of PC_{20} and eosinophils in bronchial brush biopsies (BBB) in the asthmatic patients. Spearman's rank correlation test. PC_{20}: provocative concentration of methacholine causing a 20% fall in forced expiratory volume in one second.

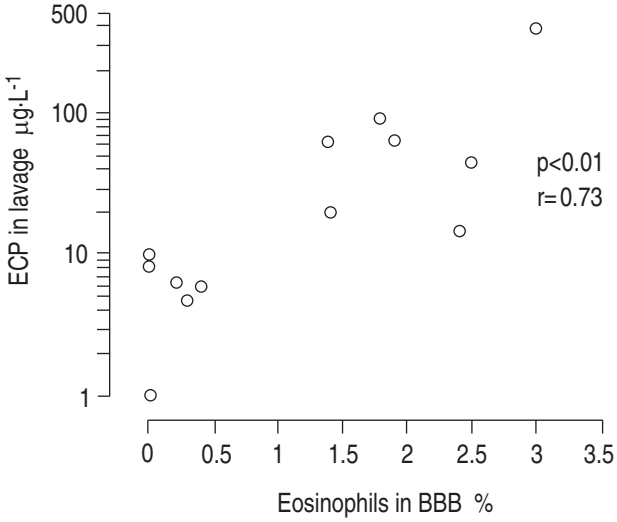

Fig. 3. – Correlation between ECP (logarithmic scale) in bronchial lavage fluid and eosinophils in bronchial brush biopsies (BBB) in steroid-untreated asthmatic patients. Spearman's rank correlation test. ECP: eosinophils cationic protein.

Table 3. – Small volume bronchial lavage levels of inflammatory cell markers in each subject group

Group		ECP $\mu g \cdot L^{-1}$	MPO $\mu g \cdot L^{-1}$	HA $\mu g \cdot L^{-1}$	IL-8 $ng \cdot L^{-1}$	Tryptase $U \cdot L^{-1}$
1.	Healthy controls	3.8 (0–34)	120 (0–3147)	10 (0–599)	167 (48–6320)	2.9 (0–20)
2a.	Asthma without inhaled steroids	10.0* (0–318)	85 (30–1345)	46** (12–102)	200 (54–955)	4.4 (0–8.2)
2b.	Asthma with inhaled steroids	11.0* (2.8–96)	128 (10–452)	32* (0–116)	295 (53–1915)	2.3 (0–17.5)
3.	Chronic bronchitis	14.1 (0–116)	71 (0–3108)	23* (14–208)	398 (5–23308)	1.1 (0–5.8)

Values are presented as median, and range in parenthesis. MPO: myeloperoxidase; HA: hyaluronan; IL-8: interleukin-8. *: $p<0.05$; **: $p<0.01$; *versus* the controls (Mann-Whitney U-test).

Table 4. – Serum levels of inflammatory cell markers in each subject group

Groups		ECP $\mu g \cdot L^{-1}$	MPO $\mu g \cdot L^{-1}$	HA $\mu g \cdot L^{-1}$	IL-8 $ng \cdot L^{-1}$
1.	Healthy controls	6.2 (2.1–18.6)	395 (300–690)	24 (0–54)	0 (0–3780)
2a.	Asthma without inhaled steroids	10.5* (2.3–41.0)	415 (270–610)	11 (0–47)	0 (0–137)
2b.	Asthma with inhaled steroids	7.8 (4.1–14.0)	405 (240–515)	20 (0–51)	0 (0–962)
3.	Chronic bronchitis	9.8 (4.1–24.1)	345 (270–860)	29 (0–186)	0 (0–436)

Values are presented as median, and range in parenthesis. *: $p<0.05$ *versus* the controls (Mann-Whitney U-test). For definitions see legends to table 3.

The mean levels of MPO, IL-8 and tryptase in bronchial lavage and in serum were similar between the asthmatic patients (Group 2) and the healthy controls (tables 3 and 4).

The subjects with atopy (n=23) had significantly higher mean bronchial lavage levels of ECP (47.5 $\mu g \cdot L^{-1}$) than those without atopy (15.5 $\mu g \cdot L^{-1}$), ($p<0.05$), but their serum ECP levels did not differ significantly. The bronchial ECP levels correlated significantly with the ECP serum levels ($r=0.46$; $p<0.05$) in the atopic patients (data not shown).

The bronchitic patients (Group 3) had a significantly higher mean bronchial lavage level of hyaluronan ($p<0.05$) than the controls (table 3). The mean levels of ECP, MPO, IL-8 and tryptase in bronchial lavage and in serum were not significantly different between the bronchitis patients and the healthy controls (tables 3 and 4).

The levels of tryptase in serum were all below the level of detection (data not shown).

Highly significant correlations were found between the bronchial lavage levels of ECP and hyaluronan ($r=0.68$; $p<0.001$, Spearman's rank test), and between the bronchial lavage levels of ECP and IL-8 ($r=0.74$; $p<0.001$). Also, bronchial lavage hyaluronan correlated significantly with age ($r=0.55$; $p<0.01$).

No significant correlations were found between the levels of inflammatory markers and the clinically measured parameters of FEV_1, PC_{20}, duration of symptoms or age (except for hyaluronan, as shown above).

Discussion

Recently, we presented standardized bronchial brushings as a reproducible and relatively simple technique to study airway epithelial cells morphologically in airway disease [1]. Since then, others have found the method useful both for morphological [2] and functional [3, 4] studies of airway cells, including culturing of epithelial cells [28]. In the present study, we used fibreoptic bronchoscopy and the BBB method to study epithelial inflammatory cells in the two inflammatory airway diseases, bronchial asthma and chronic bronchitis. The degree of activation of bronchial inflammatory cells was evaluated indirectly by measurement of cell activation markers in small volume bronchial lavage fluid, a method known to give samples representative of the large airways [29–31]. Bronchial lavage can be performed with the bronchoscope in a wedged [29, 30] or unwedged position [31], and we chose the latter to further minimize possible contamination from the alveolar space. We found that the BBB method was well-tolerated, and gave both intelligible and credible results.

An increased percentage of eosinophil granulocytes was found in the brush biopsies from the steroid-untreated asthmatic patients in comparison with the other groups. The single subject with high eosinophil percentage among the controls was an atopic, who developed asthma within 1 year after the study. These findings are in accordance with results from studies using bronchial biopsies obtained by forceps to investigate the eosinophilic airway inflammation of asthma [5–9]. In addition, the BBB method gives results related to the clinical state of asthma shown by the significant negative correlation for the percentage of eosinophils with the levels of PC_{20} for the asthmatic patients in the study. This reflects the known relationship between bronchial eosinophilic inflammation and degree of airway reactivity in asthma [32, 33]. Our results are comparable to those of GIBSON *et al*. [2], who reported that a group of steroid-untreated asthmatics

had eosinophil values between 0.2 and 6.7% in bronchial brush biopsies, whereas the nonatopic controls had none. However, their subject groups were smaller (23 subjects in four groups), and the differences reported were not statistically significant.

The cytological findings in the BBB method appear to be associated with airway inflammatory cell activity. A significant correlation between the percentage of eosinophils in brush biopsies and ECP levels in bronchial lavage indicates that the BBB method reflects the degree of eosinophil granulocyte activation measured as ECP [10]. The correlation was especially convincing for the steroid-untreated asthmatic group (fig. 3). The percentage of neutrophils in brush biopsies was found to correlate significantly with MPO, a marker of activated neutrophil granulocytes [34], and the cytokine IL-8, which has a known chemotactic effect on neutrophil granulocytes [32].

However, these last two correlations were weak, and of uncertain clinical relevance. Our results indicate that a quantitative analysis of eosinophils, and possibly neutrophils, in BBB could be of use when studying bronchial changes in asthma as well as other inflammatory airway diseases, *e.g.* chronic bronchitis as well as chronic obstructive pulmonary disease, where neutrophil inflammation is pronounced [19, 35].

As expected, the steroid-untreated asthmatic patients in the study had increased levels of ECP both in small volume bronchial lavage and in serum as compared with the healthy controls. The asthmatic patients given medication in the form of regular steroid inhalation had a significantly increased level of ECP in bronchial lavage, but not in serum. This is in agreement with other reports, reflecting the greater eosinophil activity in the asthmatic patients not given treatment with steroid inhalation [14, 15]. The levels of tryptase in bronchial lavage were comparable between the groups, probably because all asthmatics were investigated during a stable phase in their disease and the bronchoscopies were performed outside the pollen season. In agreement with the findings by LINDEN *et al*. [20], the median bronchial lavage ECP levels were highest for our patients with nonobstructive chronic bronchitis, but this value did not differ significantly from the other groups due to a large spread in data and small number of subjects. LACOSTE *et al*. [36] reported increased eosinophils and low levels of ECP in patients with chronic bronchitis, but they analysed BAL fluid, and the changes were significant only for the obstructive bronchitic patients. The levels of MPO in our patients were similar to earlier findings [20], but lower than those reported by LACOSTE *et al*. [36] in BAL fluid. It is possible that the investigation of bronchitic subjects in whom there is an obstructive component and/or recurrent infectious exacerbations could yield different results than in our nonobstructive and noninfected chronic bronchitis patients [35].

It appears that the lavage method can provide an advantage over bronchial biopsies by reflecting activation of cells normally not easy to quantitate in biopsies, *e.g.* hyaluronan from activated fibroblasts. The increased levels of hyaluronan found in all the patient groups in the present study are, however, difficult to interpret. Hypothetically, it could reflect the process of subepithelial fibrosis leading to a thickening of the reticular basement membrane known to be present in asthma [5, 6]. It could also reflect a nonspecific inflammation present both in asthmatic and bronchitic patients, as indicated by the significant correlations for hyaluronan with both age and ECP.

To conclude, our results indicate that the bronchial brush biopsy method gives results which relate to the degree of inflammatory activity and disease severity in asthma. The method yields additional information about the superficial bronchial epithelial changes in inflammatory airways disease. It can possibly be of clinical value in the investigation of cases with unexplainable cough or dyspnoea, where an increased finding of eosinophils would indicate latent asthmatic disease.

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